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A thesis submitted in partial fulfilment of the requirements for the Degree of Master of Applied Science at Lincoln University by Mauricio Alvaro Urrutia Correa

Lincoln University
2005


By M. A. Urrutia Correa

The Argentine stem weevil, Listronotus bonariensis (Kuschel) (Coleoptera: Curculionidae), is the most important pest species in New Zealand, costing the pastoral industry an estimated NZ$78-251 m per year (Prestidge et al., 1991). The parasitoid wasp Microctonus hyperodae Loan (Hymenoptera: Braconidae) was released in New Zealand in 1991 as a potential biological control agent of the weevil (Goldson et al., 1994). As M. hyperodae is a provovigenic species, its lifetime complement of eggs is set at adult emergence (Phillips, 1998). Adult wasp fitness is likely to be affected by host nutrition, as resources carried over from immature stages to the adult stage of parasitoids can vary with the size and nutritional quality of the host (Jervis et al., 2001). The fecundity of Argentine stem weevil can increase after the addition of pollen to its diet (Evans and Barratt, 1995). Conversely, a decrease in gonad development (i.e., vitellarium development and percentage of females with oocyte resorption) can occur after consumption of ryegrass containing endophyte (Barker and Addison, 1996). This research investigates tri-trophic-level interactions on parasitoid fitness (i.e., egg load and body size) by assessing field-collected adult weevils, and by providing bee-collected pollen, buckwheat (Fagopyrum esculentum Moench cv. Katowase) pollen, ryegrass-only (Lolium multiflorum L. cv. Tama), or ryegrass (Lolium multiflorum Lambrechtsen cv. Aries) containing endophyte (Neotyphodium lolii (Latch, Christensen and Samuels) Glenn, Bacon and Hanlin in the host diet in the laboratory.

A survey was conducted in the Lincoln area to what extent pollen was part of the diet of field-collected Argentine stem weevils. The frequency and types of pollen grains consumed were
analysed. Also, gut fullness (in terms of ryegrass particle content), parasitoid larval presence, weevil gender and weevil gonad development were also assessed. Argentine stem weevil adults fed on pollen in the field, although a low proportion of them contained pollen in their gut. Gonad development was positively correlated with the extent of pollen consumption (i.e., number of individuals with sexually mature gonads).

A cage experiment was conducted in the laboratory. Potentially parasitised adult Argentine stem weevils were collected from ryegrass on roadsides in the Lincoln area during 2004. They were randomly allocated to four treatments: ryegrass-only (control), ryegrass plus bee-collected pollen, ryegrass plus buckwheat pollen, and ryegrass with endophyte, each replicated four times. Water was provided in every treatment. The parasitoids that emerged were assessed for size (i.e., hind leg tibial length) and potential fecundity (i.e., egg load) (see Phillips and Baird, 2001). Despite the fact that the addition of bee-collected or buckwheat pollen to the host diet had no significant effect on the number of adult parasitoids emerged, their body size or egg load ($P > 0.05$), some factors that could have affected a potentially positive correlation between parasitoid fitness and pollen consumption are discussed. Similarly, the addition of ryegrass with endophyte had no significant effect on the number of adult parasitoids emerged, their body size or egg load ($P > 0.05$). This leads to the suggestion that biological control (i.e., $M. hyperodae$) and plant resistance (i.e., endophyte) strategies can work in a complementary way in integrated pest management programmes.

The concept of benefiting the third trophic level with 'resource subsidies' made available selectively to the second level has the potential to lead to the suggestion of a new mechanism for enhancing parasitoid fitness, that is 'indirect' conservation biocontrol.

*Keywords*: Argentine stem weevil, *Listronotus bonariensis*, *Microctonus hyperodae*, ryegrass, buckwheat, pollen, endophyte, conservation biological control, tri-trophic interactions.
To my wife and daughters............

“........far from being a purely passive victim, obliterated without a trace, the host is often able to impress its mark ...... upon the insect parasitoid that destroys it” (Salt, 1941).
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CHAPTER 1

GENERAL INTRODUCTION

BIOLOGICAL CONTROL BACKGROUND

Early agricultural production systems were probably designed partly to maximise the impacts of the natural enemies of the herbivores that were attacking crops. For example, in ancient China, colonies of ants were introduced into citrus groves to control pests (Gurr et al., 2000a). However, it was around 1900 when the first major episode of biological control of an arthropod by an arthropod occurred; it was the control of cottony-cushion scale (Icerya purchasi Maskell; Homoptera: Margarodidae) on citrus in California following the importation of a predator, the vedalia beetle (Rodolia cardinalis (Muls)), from Australia (Doutt, 1964).

However, biological control of arthropods by arthropods has its problems and critics (Gurr et al., 2000b). A success rate of only 10% has been recorded in classical biological control, with no major improvement occurring with time (Greathead and Greathead, 1992). This is mainly due to a "shot-gun" approach of releasing as many species of agents as were available in the hope that control would be achieved. However, the intentional introduction of alien species into complex biological communities is a threat to their structure and dynamics (Louda et al., 2003). Indeed, any method that reduces the population of an abundant pest organism to below an economic threshold will have inherent environmental risks (Simberloff and Alexander, 1998; Howarth, 2000). Because the release of non-indigenous agents to control pests is usually irreversible, and because the introduced agents can attack non-target organisms, reproduce, evolve, and spread away from the point of release, their potential for causing damage is high (Simberloff and Alexander, 1998; Howarth 2000; Louda et al., 2003). Subsequently, there has been increasing emphasis on pre-release experimentation to select the best agents and increase the subsequent likelihood of success (Gurr et al., 2000b). Also, environmental and economic costs of inaction and of alternative actions must be weighed before the release of a biocontrol agent (Frank, 1988).
The first attempt at biological control of Argentine stem weevil, *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae), in New Zealand was made in the 1960s. The Department of Scientific and Industrial Research introduced an egg parasitoid, *Patasson (Anaphes) atomarius* (Brethes) (Hymenoptera: Mymaridae) as a potential biological control agent, but it failed to establish (Dymock, 1989). In 1991, the South American parasitoid wasp *Microctonus hyperodae* Loan (Hymenoptera: Braconidae) was released in New Zealand as a potential biological control agent of the weevil and, within three years, maximum parasitism rates of >90% were being recorded (Goldson et al., 1994a). *M. hyperodae* oviposits in adult Argentine stem weevils, and a solitary parasitoid larva develops within the living, active host. The mature larva then emerges to pupate, while the host dies as a result of the parasitism (Phillips and Baird, 2001).

**THE ARGENTINE STEM WEEVIL AND CURRENT CONTROL PRACTICES**

**BACKGROUND**

**Argentine stem weevil**

Argentine stem weevil was first recorded in New Zealand in 1927 (Marshall, 1937), and was first recognised as a pest of wheat in 1933 (Pottinger, 1961a). It was not until the late 1950s that this weevil’s potential for damaging pasture was recognised (Pottinger, 1961b). Since then, it has become regarded as New Zealand’s most damaging pest of ryegrass (*Lotium* spp.), and before the introduction of a biological control agent, was estimated to cost the pastoral industry between NZ$78-251 m per year (Prestidge et al., 1991). Pasture covers over 10 million hectares (38%) of New Zealand and is the largest single land use in the country (Land Cover Data Base, 1997). Perennial ryegrass (*Lotium perenne* L.) and Italian ryegrass (*Lotium multiflorum* Lambrechtsen) are the most widely used pasture species (Charlton and Stewart, 2000).

Argentine stem weevil is a South American species and is widespread in Argentina, Uruguay, Chile, Bolivia and Patagonia (Barker and Pottinger, 1982). It has been recognised as a minor pest in Tasmania and New South Wales (Australia) (Barker and Pottinger, 1982). Its status as a key or primary pest in New Zealand (Barker and Pottinger, 1982), but not being a notable problem elsewhere, is similar to several other species (Goldson et al., 1998b). Goldson et al.
(1997) attributed such eruptions of relatively minor pests to a lack of natural enemies in New Zealand's highly modified pastoral ecosystem.

In New Zealand, the weevil is summer-active (Goldson et al., 1998a) and has two complete generations each summer in the South Island (Barker and Pottinger, 1982), though three may occur in the warmer parts of the North Island (Pottinger, 1961a). In 1982, Barker and Pottinger (1982) observed that eggs and larvae of the first summer generation were considerably more abundant than those in the second. However, by 1995-96, this trend had disappeared and this coincided with a growing impact of *M. hyperodae* on the overwintering survival and reproductive capability of the weevil, from the 1993-94 season onward (Goldson et al., 1998b).

The weevil overwinters in a state of photoperiodically-induced diapause (Goldson and Emberson, 1980), with spring egg-laying commencing in late September (Goldson et al., 1998b). By the end of December, the remaining now senescent overwintered population has died and is replaced by the first summer generation (Goldson et al., 1998b). Oviposition by the first summer generation peaks in early February, with second-generation adult emergence starting in early March and persisting until mid June (Goldson et al., 1998b).

**Graminaceous crops and Argentine stem weevil in New Zealand**

Argentine stem weevil is a major pest of graminaceous crops in New Zealand (Barker et al., 1984), and attacks maize, sweetcorn (Barker et al., 1988b), wheat, barley and oats, as well as most pasture grasses (Timlin, 1964). However, the weevil shows marked preferences for some species such as short-rotation ryegrass (*L. multiflorum* hybrid) and Italian ryegrass (*L. multiflorum*) (Timlin, 1964).

In ryegrass, the female weevil deposits 1 - 8 eggs in the leaf cuticle (Pottinger, 1961a; Goldson et al., 1998b); then the newly hatched larvae typically tunnel downward within the tiller (Plate 1) (Barker et al., 1984). More than one larva may feed on a tiller, particularly during the early instars (Barker et al., 1984). Later, the resulting stem-mining larvae feed on 3-8 tillers of ryegrass before pupating in the soil (Goldson et al., 1998b).

There are three types of damage caused by the weevil on ryegrass: a) feeding by adults gives a silvery appearance to leaves, but recovery from this type of damage is usually good (Kelsey,
However, the survival of summer-sown ryegrass seedlings may be reduced by adult feeding (Chapman, 1984), b) the second and most severe damage is caused by the larvae tunnelling in tillers (Kelsey, 1958; Pottinger, 1961a), c) the third type of damage is to seed heads. When these are being produced, larvae nearly always move upwards in stems and the result is a “white head” that does not set seed (Kelsey, 1958).

Generally, newly-established pastures (<5 years-old) are most favourable for the weevil and are most prone to damage. There have been a number of estimates of damage levels, either by direct examination of tillers or by measuring pasture production in insecticide-treated and untreated areas (Barker et al., 1984). Based on tiller dissections, damage to annual ryegrass has been as high as 98%, whereas perennial ryegrass suffered 30-40% damage (Barker et al., 1984).

The weevil is also a major problem in seedling maize sown soon after the cultivation of pasture, or in previously-cropped ground where grass weeds, including Poa annua L. and couch, Elytrigia repens (L.) Beauv., are present. The weevil is the greatest cause of seedling losses in maize in the Waikato region of the North Island of New Zealand (Watson and Hill, 1985).
Plate 1. *Listronotus bonariensis* larva. Ryegrass tiller was cut longitudinally (Photo from AgResearch, used with permission).

Plate 2. *Microctonus hyperodae* ovipositing on *Listronotus bonariensis* (Photo from AgResearch, used with permission).

Plate 3. *Microctonus hyperodae* larva inside female *Listronotus bonariensis*.

Plate 4. *Microctonus hyperodae* larva emerging from *Listronotus bonariensis* (Photo from AgResearch, used with permission).
Management tools for Argentine stem weevil

Chemical control
Over the past 40 years there has been continual investigation into the use of insecticides to control Argentine stem weevil. However, the mining habit of the larvae coupled with considerable flight activity of the adults (Goldson, 1981a), especially in dry east coast areas of the South Island of New Zealand, make cost-effective chemical control unlikely (Goldson et al., 1998a). When establishing pastures, drilling with a systemic granular insecticide (Chapman, 1984) or using seeds treated with chemicals (Goldson et al., 1994b) can offer protection to the seedling. However, in established pasture, control by insecticides is highly variable (Chapman, 1984), with increases in pasture yields due to Argentine stem weevil control from 0% to 91% in the first spring-autumn period of the pasture (Barker et al., 1984).

Cultural control
Good pasture management may also assist in alleviating the effects of weevil damage. Some aspects of pasture management that may be manipulated for pest control include the timing and method of establishment, the timing and intensity of grazing or cutting, and the use of fertiliser and irrigation (Gregg, 1997).

Time of sowing of crops may be important in limiting damage. Spring-sown grasses and cereals are more severely affected than autumn-sown crops. In some areas of New Zealand, December-January-sown ryegrass crops have suffered heavy second-generation attack due to both adult and larval feeding (Pottinger, 1961a; Matthews et al., 1999). Sowing of grain maize and sweet corn in South Auckland extends from early October to mid December. Crops sown at this time are particularly at risk unless sowing is preceded by adequate cultivation to eliminate previous pasture residual populations (Barker et al., 1988a). Independent of plant species, young plants are more susceptible and do not have the same ability to recover as mature plants (Pottinger, 1961a; Matthews et al., 1999). In addition, ploughing in spring can reduce weevil larvae populations up to 70%, whereas autumn ploughing is less effective because larvae are less susceptible to injury than pupae (Matthews et al., 1999).

Grazing livestock, for instance, affects pests by trampling or ingesting them, or by changing the microclimate or food supply (Gregg, 1997; Matthews et al., 1999). Cutting also removes pests and changes the microclimate, often to the detriment of the pest, though damage to natural enemies may lead to a reduction of biological control (Gregg, 1997). Fertiliser
application and irrigation generally has little direct effect on pests, but can allow pastures to recover more rapidly from insect attack (Gregg, 1997; Matthews et al., 1999). However, they also can make plants more attractive to pests (Gregg, 1997).

On the other hand, crop rotation is another cultural control practice that involves measures such as growing potato crops after cereals and crucifers, before sowing pasture again, the idea being to eliminate the existing population of weevils before sowing the next susceptible crop (Pottinger, 1961a; Watson and Wrenn, 1978; Barker et al., 1988a). If maize is to be sown in land that was previously in permanent pasture or short rotation ryegrass winter green feed, a fallow of 4 - 6 weeks between cultivation and drilling the maize should be allowed (Chapman, 1984).

**Plant resistance**

Ryegrass may contain an endophytic fungus, *Neotyphodium lolii* (Latch, Christensen and Samuels) Glenn, Bacon and Hanlin (Keogh, 1973), which produces three major toxins within the plant, especially near the shoot base and seed-heads: a) peramine, which deters feeding and oviposition by the weevil and some other pests, b) lolitrem B, that causes “ryegrass staggers” in grazing animals and c) ergovaline that causes heat stress and blood circulation problems in grazing animals (Charlton and Stewart, 2000). Harm to animals can be diminished by using mixtures of ryegrass with endophyte-free cultivars or other pasture species, or by using low-endophyte cultivars, which are used especially for deer and horses, because these animals are more sensitive to ryegrass staggers (Charlton and Stewart, 2000).

A high-endophyte cultivar has an endophyte percentage over 70% and a low endophyte cultivar contains about 5% of the fungus (Charlton and Stewart, 2000). The endophyte AR1 produces peramine that provides resistance to the weevil but lacks the mammalian toxins ergovaline and lolitrem B (Popay and Baltus, 2001). However, factors such as host plant genotype and variations in hyphal content in individual plants, as well as infection by other foliar endophytes and preferences amongst individual weevils may influence the level of resistance. Moreover, the level of resistance is significantly reduced in the presence of infection by the mycorrhizal fungus *Gliocladium fasciculatum* (Barker, 1987).
**Biological control**

*Microctonus hyperodae as a biological control agent*

*Microctonus hyperodae* was imported from eight South American locations in Argentina (Ascasubi, Mendoza, General Roca and S.C. de Bariloche), Brazil (Porto Alegre), Chile (Concepcion and La Serena) and Uruguay (Colonia) (Goldson et al., 1990; Fig 1). Quarantine-based host specificity testing suggested *M. hyperodae* was oligophagous, and the results indicated it was unlikely to threaten New Zealand species of Curculionidae (Goldson et al., 1992).

Permission to release the parasitoid was granted by the Ministry of Agriculture and Fisheries (Goldson et al., 1992) and the parasitoid was released in the form of parasitised weevils (Barlow et al., 1994) at nine sites throughout New Zealand during 1991 (McNeill et al., 1993; Fig. 1), in approximately equal numbers of each South American geographic population (Phillips et al., 1996). After release, the parasitoid species successfully established in Canterbury and Northland (McNeill et al., 1993).

Based on models, high parasitism rates (i.e., > 60%), are a significant estimate of the probability that a parasitoid introduction will reduce host densities (Hawkins et al., 1993; Hawkins and Cornell, 1994; Williams et al., 1994). Hawkins and Cornell (1994) noted that there is a threshold for success in the introduction of a parasitoid; when the parasitism rates are below 32% in the native range, no control of the pest is achieved with the parasitoid. Moreover, Myers et al. (1994) stated that host parasitism rates below 30% in the native range are significant estimates that the host will not be amenable for biological control using parasitoids.

Once introduced to New Zealand, *M. hyperodae* achieved parasitism rates that exceeded 80% in some cases. This parasitoid was liberated between 1991 and 1993, at 12 New Zealand locations and established itself at 10 of the sites (Goldson et al., 1994a). Today it is established at over 80 locations in the North and South Island (Phillips et al., 1998), being widely distributed throughout the North Island and in more limited coverage across the South Island (mainly Canterbury Plains) (McNeill et al., 2002a).
Biology

*Microctonus hyperodae* was first found in 1961 as a parasitoid of adult weevils at Colonia, Uruguay, where four specimens emerged from 750 *Hyperodes (=Listronotus) bonariensis*, which is a native species of southern South America (Loan and Lloyd, 1974). Loan and Lloyd (1974) were the first to describe the taxonomy and biology of this parasitoid, and its development within its host. They found that only females emerged from parasitised weevils, indicating that the species is thelytokous (Loan and Lloyd, 1974).

*Microctonus hyperodae* females oviposit between 30 and 60 eggs (McNeill et al., 1993) laying one egg per weevil in several adult weevils (Barlow et al., 1994; Plate 2) and reach the maximum egg laying potential at 24 hours old (Phillips et al., 1996). The larva of this solitary endoparasitoid develops within the living host until maturity (McNeill et al., 1993; Phillips et al., 1996; Plate 3). The mature larva then emerges to pupate, while the host dies as a result of the parasitism (Phillips et al., 1996; Plate 4).
During winter, the parasitoid enters photoperiodically-induced diapause independent of the host condition (McNeill et al., 1993; Barlow et al., 1994) as an arrested egg or larva (Barlow et al., 1994). Development resumes in the spring with increase in temperature. It probably completes three generations per year, with adults found in the field in late December through to May (McNeill et al., 1993; Barlow et al., 1994). It seems likely that each adult generation lives less than two weeks in the field, since their adult longevity under ideal conditions in the laboratory was 17 days (Phillips, 1998).

**Egg load**

In contrast to syn-ovigenic parasitoid species, where the parasitoid is able to mature eggs throughout its reproductive life (Jervis et al., 2001), *M. hyperodae* is described as a pro-ovigenic species (Goldson et al., 1995; Phillips and Baird, 2001), so its lifetime complement of eggs is set at emergence from the host. This can limit parasitoid fecundity if hosts are abundant (Heimpel and Rosenheim, 1997), because the total set of eggs can be laid before death. *M. hyperodae* adults in the field were estimated to have a mean pre-oviposition egg load of 67 eggs (Phillips et al., 1998). However, parasitoids reared in the laboratory had a mean egg load of 47 (Phillips and Baird, 2001), which corresponded well to the fecundity of 48 eggs estimated by Goldson et al. (1995). Furthermore, when parasitised weevils are collected in the field and maintained in the laboratory, the emerging parasitoid adults also had a mean egg load of 47 eggs (C.B. Phillips, personal communication, February 2003). This variation between field-collected and laboratory-reared parasitoids suggests unidentified environmental factors have a major influence on parasitoid egg load. Identifying such factors is the main goal of the research of this thesis. Indeed, models have predicted that the fecundity of a parasitoid may have less influence on prey density than its searching ability, although egg load may be far more amenable to enhancement than searching efficiency. Subsequently, parasitoids with higher egg loads would be potentially good candidates for reducing prey populations (Kean et al., 2003).

Host nutrition is likely to have an effect on the ability of a parasitoid to develop normally (Beckage and Riddiford, 1983). The weevils used in the laboratory experiments of Phillips and Baird (2001) were fed only ryegrass (C.B. Phillips, personal communication, February 2003), but weevils in the field may have access to additional food sources. For example, pollen is a rich source of nutrients and enhances, in the laboratory, the survival, body fat, gonad development, and reproduction of boll weevil (*Anthonomus grandis* Boheman;
Coleoptera: Curculionidae) in Mexico (Jones et al., 1993), and eggs per female of Argentine stem weevil in New Zealand (Evans and Barratt, 1995).

In parasitoid wasps, the resources carried over from the larval to the pupal and adult stages vary with the size and nutritional quality of the host and the time available for larval development (Beckage and Riddiford, 1983; Harvey et al., 1995; Jervis et al., 2001). This carry-over of resources from the host is positively correlated with wasp body size within parasitoid wasp species (Harvey et al., 1995; Jervis et al., 2001), and adult body size is generally correlated with egg load at emergence (Jervis et al., 2001). In this way, the type of food consumed by hosts could influence the reproductive success of their parasitoids (Harvey et al., 1995). However, *M. hyperodae* is an unusual case where some geographic populations (e.g., those from Chile and from S.C. de Bariloche in the Argentinian Andes) exhibit relatively strong correlations between body size and egg load, yet other geographic populations from east of the Andes do not (Phillips and Baird, 2001).

Following these ideas, egg load variation between *M. hyperodae* adults collected in the field and those reared in the laboratory could be due to differences between the diets of host weevils in the field and laboratory. Improving the understanding of the environmental factors which influence egg load could assist biological control in several ways. For example, the rate of establishment of newly-introduced biological control agents might be improved by providing conditions that optimise parasitoid egg load both during laboratory mass rearing and in the field during and after parasitoid release. Knowledge of environmental effects on parasitoid egg load might also support the development of new habitat manipulation strategies to enhance the suppressive effects of established parasitoids on pest populations. For example, provision of plants that produce pollen available to weevils at times of the year when weevil adults contain parasitoid larvae could result in adult parasitoids with increased egg loads, thus potentially enhancing parasitism rates. Another possible approach involves augmentation biological control. For instance, parasitised Argentine stem weevils, which had been fed pollen, could be released in organic sweet corn fields. The adult *M. hyperodae*, which emerged from the pollen-fed weevils, might achieve higher parasitism rates than those parasitising weevils that had fed on only ryegrass.

To achieve successful biological control there is a need for selective floral resources (Wratten et al., 2004). Wratten et al. (2004) stated that selective floral resources have a hierarchical
component. This is: 1) parasitoid benefits but pest does not. 2) parasitoid and pest benefit, but the pest has higher longevity only; 3) parasitoid and pest benefit but parasitoid benefits relatively more; and 4) parasitoid and pest benefit but the improved fitness of the pest leads to a further improvement in the parasitoid’s fitness because the developing parasitoid larva derives a higher level of nutrients from a host that has fed on, e.g., pollen (Wratten et al., 2004). The last of these possibilities represents the novel concept of “indirect resource subsidies”, which was exemplified previously.

GOAL AND OBJECTIVES

The goal of this study is to elucidate effects of host diet on parasitoid fitness to increase knowledge of plant/parasitoid/host interactions.

Objective 1. To determine if the addition of bee pollen to the weevil’s diet influences parasitoid egg load and parasitoid body size, and to measure the relationship between parasitoid body size and parasitoid egg load.

Objective 2. If the addition of pollen to the weevil’s diet influences parasitoid egg load, then Objective 2a will further examine in the laboratory the effects of different components of the weevil diet (i.e., buckwheat pollen) on parasitoid egg load, survival, and reproduction (i.e., oviposition rate). In addition, weevils captured from the field will be assessed for the amount and types of pollen within their guts.

If pollen does not influence parasitoid egg load, then Objective 2b will further examine in the laboratory the effects of other components of the weevil diet (i.e., endophyte ryegrass and buckwheat pollen) on parasitoid egg load. In addition, weevils captured from the field will be assessed for the amount and types of pollen within their gut.

HYPOTHESES

1. Parasitoids reared from weevils fed with pollen and ryegrass will have higher egg loads than those reared from weevils fed only with ryegrass.

2. Parasitoids reared from weevils fed with no-endophyte ryegrass (without pollen) will have higher egg loads than those reared from weevils fed endophyte ryegrass (without pollen).

3. Weevils collected from the field will have pollen in their gut.
CHAPTER 2

GUT CONTENTS OF FIELD-COLLECTED ARGENTINE STEM WEEVILS (*Listronotus bonariensis* (Kuschel)) (COLEOPTERA: CURCULIONIDAE)

INTRODUCTION

Argentine stem weevil feeds on the leaves of plants of the family Poaceae (Morrison, 1938; Kelsey, 1958; Barker *et al.*, 1989). However, pollen is also a food source for this weevil (Evans and Barratt, 1995) and for at least four other species from the same family (i.e., Curculionidae) (Haegermark, 1980; Nanda and Pajni, 1991; Jones *et al.*, 1993; Gultekin *et al.*, 2003).

After weevil feeding activity, pollen can be found externally on an insect’s cuticle and/or internally in the digestive system (i.e., gut). Several authors have examined the pollen found on the exoskeleton of insects to assess their cuticle pollination capacity (de los Mozos and Martin, 1988), or migration patterns (Gregg, 1993; Silberbauer *et al.*, 2004). Others have examined the pollen content of the gut of insects to assess diet breadth (Rummel *et al.*, 1978; Jones *et al.*, 1993; Hickman *et al.*, 1995; Irvin *et al.*, 1999), migration patterns (Del Socorro and Gregg, 2001), or habitat manipulation management strategies (Lövei *et al.*, 1993; Wratten *et al.*, 1995; Hickman and Wratten, 1996; Silberbauer *et al.*, 2004).

Pollen analysis of field-collected insects has become an important tool in the study of insect biology and ecology because pollen grains are distinctive and can often be identified to genus (Wratten *et al.*, 1995; Del Socorro and Gregg, 2001), even after being ingested by the insect (Gregg, 1993; Wratten *et al.*, 1995; Del Socorro and Gregg, 2001; Hickman *et al.*, 2001; Silberbauer *et al.*, 2004). Also, assessing pollen as a self-marking material is inexpensive (Silberbauer *et al.*, 2004).
Pollen is also a rich source of nutrients and its consumption by insects, either in the laboratory or in the field, can increase their fecundity (Pesho and van Houten, 1982; Annis and O'Keeffe, 1984; Jones et al., 1993; Evans and Barratt, 1995; Hickman and Wratten, 1995; Irvin et al., 1999; Landis et al., 2000). However, pollen consumption can vary between genders (Irvin et al., 1999; Takakura, 2004), and can influence the consumption of other components of the insect diet (Evans and Barratt, 1995; Barratt et al., 1996). Furthermore, in theory, pollen consumption could influence the nutritional quality of the host and hence make it more suitable for parasitism, compared with a host which has not fed on pollen.

It was hypothesised that pollen is part of the Argentine stem weevil diet and that its consumption would vary depending on seasonal availability. It was also hypothesised that pollen consumption would increase the proportion of sexually mature weevils, and decrease ryegrass intake in both genders. Finally, it was hypothesised that weevils that fed on pollen would constitute better quality hosts and consequently generate parasitoids with greater fitness.

In order to assess these hypotheses, an experiment was designed with the objective of analysing the frequency and types of pollen grains consumed by field-collected weevils. Gut fullness, parasitoid presence, weevil gender, weevil gonad development and the interactions of all these variables with pollen consumption were also assessed.

MATERIALS AND METHODS
Argentine stem weevil adults were collected from ryegrass pastures in the Lincoln area on five dates (December 12th, 2003 and February 11th, March 26th, June 15th, and September 30th of 2004), using a sweep net. As soon as possible after capture (generally within 4 hours), the weevils were separated from the debris and frozen at -80°C.

1. Pollen content
To assess the pollen content of weevils' gut, the weevils were washed for at least one minute, by shaking them with water and detergent, to eliminate pollen grains present on the exoskeleton of the insects (J. Martin, personal communication, November 2003). Then the weevils were dissected on dark purple paraffin wax plaques moulded in 9 cm diameter plastic Petri dishes. Each weevil was secured for dissection by pressing its ventral surface into a
small area that had been melted using a hot needle. All work was performed under a microscope at 20X, 40X or 63X magnification. Using a pair of fine forceps, the elytra were lifted off, the folded wings were removed, and the dorsal surface of the abdomen was opened and pulled back. This revealed the gut, overlying the gonads. The gut was removed after severing its anterior and posterior ends and placed on a slide. Then, the method of Wratten et al. (1995) was used, which is described below.

The gut was spread over part of the slide, then a drop of saffranin (i.e., 0.1% w/v), and a drop of heated jelly were added, followed by a coverslip. The jelly comprised 7 g gelatine, distilled water to 19 ml, 33 g 82% glycerine, and 1 g phenol crystals. The slides were subsequently scanned under a microscope at 63X magnification. Pollen numbers were estimated on a semi-quantitative scale, without detailed counting at the higher pollen categories, into one of seven frequency classes (1 = no pollen grains, 2 = 1-10 grains, 3 = 11-100 grains, 4 = 101-1000 grains, 5 = 1001-3000 grains, 6 = 3001-5000 grains, 7 > 5000 grains). To prevent pollen contamination between samples, the forceps and needles were sterilised using alcohol and a Bunsen flame after each dissection. All pollen was identified at least to family level. The percentage of each pollen type per gut was also estimated from the average of counts from nine graticule quadrants (Wratten et al., 1995).

2. Ryegrass particle content
The presence or absence of ryegrass in the gut was also recorded. At the same time as gut pollen content was assessed, gut fullness was recorded as ‘full gut’, when all of the alimentary canal was full of ryegrass particles, ‘little grass’ when ryegrass particles were present only either in the foregut or midgut, and ‘no grass’ when no ryegrass particles were observed.

3. Gonad development in Argentine stem weevil
In females, there are two ovaries located dorsolaterally in the abdominal cavity (Fig. 3). Each consists of a pair of ovarioles. In reproductively mature females, there are as many as 12 developing oocytes in each ovariole and up to 4 eggs in each calyx, which is enlarged. In males, there is one pair of testes, which are located dorsolaterally in the abdominal cavity, they are divided by sutures into 8-10 wedge-shaped testicular follicles (Fig. 2). Seminal vesicles and prostate glands are contiguous with the testes and vary in size according to the level of reproductive activity. Sexually mature males have larger testes, vesicles and glands than do sexually immature males. Weevils with enlarged calyxes (and eggs present) or with
fully-grown testes, seminal vesicles and prostate glands were considered as sexually ‘mature’ individuals. The opposite condition was considered as sexually ‘immature’ individuals.

Fig. 2. Reproductive morphology and anatomy of male and female *Listronotus bonariensis* (From: Barker, 1989). (A) Gross morphology of organs from immature male adult, dorsal view. (B) Gross morphology of organs of mature, sexually mature male adult, dorsal view. (C) Stages in the development of the ovarioles of sexually immature female *Listronotus bonariensis*, dorsal view. (D) Organs of gravid female. Legend: AC. GL. = accessory gland; Bur. Cop. = Bursa copulatrix; E. = egg; EJ. D. = ejaculatory duct; GER. = germinarium; L.O. = lateral oviduct; M.O. = median oviduct; PST. = prostate gland; R.C. = resorption crystal; SEM. VES. = seminal vesicle; SP. = spermatheca; SP. GL. = spermathecal gland; TES. = testis; V.D. = vas deferens; VIT. vitellarium.
4. Parasitism

At the same time that gonad development was assessed, the presence or absence of eggs and larvae of *M. hyperodae* was recorded.

5. Statistical analyses

A log-linear model was used to analyse the statistical significance of the interactions between the categorical variables measured in the present experiment (*P* < 0.05). The variables were analysed for second-order interactions between each other, and then for third-order interactions where significant second-order interactions were observed. Also, a Chi-squared (*X^2*) analysis was used to look for further statistical differences between pollen categories.

RESULTS

Pollen content

*Amount of pollen in the weevils’ gut*

The proportion of field-collected weevils that had consumed pollen varied significantly over the collection dates (*F*$_{16}$ = 0.043, *P* < 0.05; Fig. 3). In June (i.e., winter), the percentage of weevils that consumed pollen was 1.96%, whereas it was 5.12% in December (i.e., early summer), 6.25% in February (i.e., late summer), 10.71% in March (i.e., early autumn), and 5.92% in September (i.e., spring). Furthermore, in June no weevils contained more than 100 grains of pollen in their gut, though, in December and September, some weevils had up to 3000 pollen grains in their gut. However, there were no significant differences between the amounts of pollen consumed (*F*$_3$ = 0.62, *P* > 0.05, *X^2* = 7.33; Fig. 4). In addition, there was no significant second-order interaction between pollen content and gender (*F*$_4$ = 0.23, *P* > 0.05; Fig. 5).
Fig. 3. Percentage of Argentine stem weevils from a range of collection dates that had pollen in their gut. There were significant differences between two or more bars at $P < 0.05$. Analysis was a log-linear model of categorical data. Pair-wise comparisons of dates/categories, etc., were not possible.

Fig. 4. Percentage of Argentine stem weevils that had consumed pollen in different abundance categories. There were no significant differences between bars at $P = 0.05$. 
Fig. 5. Percentage of Argentine stem weevils that had consumed pollen in different abundance categories for both genders. There were no significant differences between genders at $P = 0.05$.

**Effects of pollen consumption on gonad development**

There was a significant second-order interaction between pollen content and gonad development ($F_4 = <0.001, P < 0.05$; Fig. 6). The proportion of weevils with sexually mature gonads increased with the number of pollen grains consumed. With 1 to 10 pollen grains, the proportion of sexually mature individuals was 78%, while 100% of weevils with more than 10 pollen grains in their gut were sexually mature. Also, there was a significant second-order interaction between collection date and gonad development ($F_3 = 0.06, P < 0.05$; Fig. 7). About 44% of weevils were sexually mature except in winter (i.e., June) when only 20% of individuals were sexually mature. Finally, there was a non-significant third-order interaction between collection date, pollen content, and gonad development ($F_{12} = 1, P > 0.05$).
Fig. 6. Percentage of sexually mature and immature Argentine stem weevils that had consumed pollen over a range of pollen abundance categories. There were significant differences between two or more bars at $P < 0.05$. Analysis was a log-linear model of categorical data. Pair-wise comparisons of dates/categories, etc., were not possible.

Fig. 7. Percentage of sexually mature Argentine stem weevils over a range of collection dates. There were significant differences between two or more bars at $P < 0.05$. Analysis was a log-linear model of categorical data. Pair-wise comparisons of dates/categories, etc., were not possible.
Gender and gonad development

There was a significant second-order interaction between gender and gonad development (F1 = <0.001, P < 0.05; Fig. 8); the proportion of individuals with sexually mature gonads varied significantly between genders. The proportions of sexually mature and immature males were similar (i.e., 18.64% and 18.99% respectively), but the proportion of sexually mature and immature females differed (i.e., 21.86% and 40.5% respectively; F4 = 0.291, P > 0.05; Fig. 9). The sex ratio did not significantly vary between collection dates.

![Percentage of Argentine stem weevils with mature/immature gonads](image)

Fig. 8. Percentage of Argentine stem weevils of both genders with mature/immature gonads. There were significant differences between bars at P < 0.05. Analysis was a log-linear model of categorical data. Pair-wise comparisons of dates/categories, etc., were not possible.
Fig. 9. Sex ratio of Argentine stem weevils over collection dates. There were no significant differences at $P = 0.05$.

**Types of pollen**

The types (i.e., pollen identified to family level) and frequencies of pollen found in the gut of field-collected Argentine stem weevils are shown in Fig. 10. Pollen from a wide range of plant families had been ingested by the weevils, the most important were Plantaginaceae, 29.8%, and Poaceae, 8.7%, of the pollen found in the gut. Also, 58.3% of the pollen grains were unidentifiable (Fig 10).

The types and frequencies of pollen found on the exoskeleton of the same weevils are shown in Fig. 11. There was a wide range of families present on the exoskeleton of the weevils, the most important were Betulaceae 32.1%, Fagaceae 8.6%, Pinaceae 11.1% and Plantaginaceae 8.6%. Also, 12.3% of the pollen grains were unidentifiable (Fig 11).
Fig. 10. Pollen types and their percentages within Argentine stem weevils’ gut.

Fig. 11. Pollen types and their percentages on Argentine stem weevils’ exoskeletons.
Ryegrass particle content

There was a non-significant interaction between pollen content and ryegrass content in the gut ($F_2 = 0.07, P > 0.05$). However, there was a significant second-order interaction between gender and ryegrass content in the gut ($F_2 = 0.02, P < 0.05$; Fig. 12). Many males (i.e., 47%) had ryegrass either in the foregut or midgut (‘little grass’ Fig. 13), and only 9.2% of males had a full gut (‘full grass’ Fig. 13). However, many females had ryegrass either in one (‘little grass’) or both (‘full grass’) foregut and midgut (i.e., 48% and 45% respectively). Furthermore, there was a significant second-order interaction between gonad development and ryegrass content ($F_2 = 0.01, P < 0.05$; Fig. 13), where weevils with sexually mature gonads generally had their gut full of ryegrass (i.e., 58% of weevils), but only 30% of sexually immature weevils had a full gut.

Fig. 12. Percentage of Argentine stem weevils from both genders with three states of gut fullness. There were significant differences between bars at $P < 0.05$. Analysis was a log-linear model of categorical data. Pair-wise comparisons of dates/categories, etc., were not possible.
Fig. 13. Percentage of Argentine stem weevils with sexually mature and immature gonads with three states of gut fullness. There were significant differences between bars at $P < 0.05$. Analysis was a log-linear model of categorical data. Pair-wise comparisons of dates/categories, etc., were not possible.

**Parasitism**

There was a non-significant second-order interaction between pollen content and parasitism ($F_4 = 0.74, P > 0.05$; Fig. 14); both parasitised and non-parasitised weevils contained the same amounts of pollen. However, there was a significant second-order interaction between collection date and parasitism ($F_3 = < 0.001; P < 0.05$; Fig. 15). The parasitism rate varied widely between collection dates, ranging from zero to 50% (Fig. 15).

*Effects of parasitism on weevil's gonad development and ryegrass intake*

There was a significant second-order interaction between parasitism and gonad development ($F_1 = < 0.001, P < 0.05$; Fig. 16), where the presence of a parasitoid larva was associated with sexually immature gonads. The percentages of sexually mature and sexually immature non-parasitised weevils was 54% and 46% respectively, while parasitised weevils had percentages of 11% and 89% respectively. Also, there was a significant second-order interaction between parasitism and ryegrass content ($F_2 = < 0.001, P < 0.05$; Fig. 17), where the presence of a parasitoid larva was associated with lower ryegrass consumption. With the presence of a parasitoid larva, 54% of weevils had ryegrass either in the foregut or midgut ('little grass', Fig. 17), whereas only 28.8% of parasitised weevils had ryegrass in both foregut and midgut ('full grass'). In the absence of parasitism, 55% of weevils had ryegrass in both foregut and midgut ('full grass', Fig. 17).
Fig. 14. Percentage of Argentine stem weevils that were or were not parasitised that had consumed pollen in several abundance categories. There were no significant differences between bars ($P > 0.05$).

Fig. 15. Percentage of parasitised Argentine stem weevils over a range of collection dates. There were significant differences between two or more bars at $P < 0.05$. Analysis was a log-linear model of categorical data. Pair-wise comparisons of dates/categories, etc., were not possible.
Fig. 16. Percentage of Argentine stem weevils with sexually mature and immature gonads that were or were not parasitised. There were significant differences between bars at $P < 0.05$. Analysis was a log-linear model of categorical data. Pair-wise comparisons of dates/categories, etc., were not possible.

Fig. 17. Percentage of Argentine stem weevils with three states of gut fullness that were or were not parasitised. There were significant differences between bars at $P < 0.05$. Analysis was a log-linear model of categorical data. Pair-wise comparisons of dates/categories, etc., were not possible.
DISCUSSION

Pollen content

Argentine stem weevil diet breadth

These results show evidence that pollen is part of the natural diet of the Argentine stem weevil. Weevils fed on pollen over all collection dates, however, the proportion of weevils that had consumed pollen was only about 6%. Evans and Barratt (1995) reported that Argentine stem weevil fed on bee-collected pollen in the laboratory, but they measured neither the percentage of weevils that consumed pollen, nor the amount of pollen taken. However, up to 34.7% of field-collected boll weevils had pollen in their gut when captured in pheromone traps (Jones et al., 1992), and this figure increased to 80% when the weevils where captured feeding on flowers (Rummel et al., 1978).

Further research is needed to explain why such a low proportion of Argentine stem weevils contained pollen. It is possible pollen may not have been available in sufficient quantities in the pasture, especially during winter. In theory, pollen could have been consumed by a large proportion of individuals, but only sporadically, and the low proportion of weevils that contained pollen in their gut was of those weevils that had consumed pollen recently. It is also possible that pollen grains could have been digested within a few hours, making it impossible or difficult to detect. Although pollen’s rigid exterior is composed of one of the most enduring protein materials, pollenium (Hagler and Jackson, 2001), Jones et al. (1993) had problems identifying pollen as a result of the boll weevils’ digestive processes, which were apparently capable of breaking down the exine wall of some pollen grains. Indeed, pollen often may only remain detectable in the gut for short periods, less than eight hours in some cases (Wratten et al., 2003). Further research should involve laboratory experiments to ascertain how long pollen remains detectable after it has been ingested.

Quantity of pollen in the weevils’ gut

The proportion of field-collected Argentine stem weevils that consumed pollen varied over the collection dates. In winter, pollen consumption was much lower compared with spring, summer and autumn (Fig. 3). These results agree with those of Cuadradro and Garralla (2000), who observed that field-collected boll weevils contained fewer pollen grains in their gut during winter compared with other seasons. The same trend was found in field-collected
hover flies, *Melanostoma fasciatum* (Macquart) and *Melangyna novaezelandiae* (Macquart) (Diptera: Syrphidae), the females of which consumed more pollen in summer than they did in winter (Irvin *et al.*, 1999). Similarly, Silberbauer *et al.* (2004) observed that one of the disadvantages of pollen when used as a marker was that pollen availability was influenced by the time of the year in which the study was conducted. Generally, pollen availability in winter is lower than that in other seasons (Silberbauer *et al.*, 2004).

There was non-significant difference in the proportion of weevils that contained different abundance categories of pollen in their gut (Fig. 4). However, there tended to be fewer weevils in the high pollen abundance categories; most weevils that had pollen in their gut had 1 to 10 grains of pollen.

It is difficult at this stage to conclude if the amount of ingested pollen was a product of deliberate or incidental consumption by the weevil. In fact, pollen in the gut of field-collected Argentine stem weevil occurs at least to some extent due to contamination of leaf surfaces on which the weevils are feeding; however, some Argentine stem weevils feed deliberately on the grass inflorescences (G.M. Barker, personal communication, September 2004). Further studies are needed to determine the nature of pollen ingestion (i.e., incidental v. deliberate) by the weevil. Further research is also needed to identify the nutritional requirements and behavioural factors involved in pollen consumption by the weevil.

The abundance of pollen in the weevils’ gut had a non-significant second-order interactions with gender. Males and females showed a tendency to eat similar amounts of pollen over all collection dates (Fig. 5). This agrees with the results for other species, such as the pea weevil, *Bruchus pisorum* L. (Coleoptera: Bruchidae), where both genders fed equally on pollen (Pesho and van Houten, 1982). Similarly, the boll weevil showed no significant difference between males and females neither in the proportion of individuals that ingested pollen nor in the mean number of pollen grains consumed per weevil (Jones, 1997).

*Effects of pollen consumption on gonad development*

Although throughout all the collection dates more male than female Argentine stem weevil fed on pollen, there was a tendency in spring that more female than male weevil consumed pollen (i.e., 77% of the weevils that consumed pollen were females). This could be explained by the need of overwintering female weevils to consume pollen during spring to mature eggs.
Indeed, the addition of bee-collected pollen to the ryegrass diet of caged Argentine stem weevil significantly increased the cumulative number of eggs laid per female (Evans and Barratt, 1995). This is consistent with the results found in the present work where there was a significant and positive correlation between pollen consumption and gonad development (Fig. 6). Even with a low amount of pollen grains consumed (i.e., 1 to 10 grains), the proportion of sexually mature individuals was 78%. Moreover, the weevils that consumed over 100 pollen grains always had mature gonads.

Similarly, Annis and O’Keeffe (1984) observed that the quantity of pollen available was very important and directly related to the proportion of female field-collected pea weevils that initiated oogenesis. Moreover, female adult aphidophagous hover flies, *M. fasciatum* and *M. novaezelandiae*, required pollen for egg maturation (Hickman and Wratten, 1996). In fact, more than 95% of all gravid female hover flies caught in pastoral areas in New Zealand and wheat fields in Hampshire (UK) had pollen in their gut (Hickman and Wratten, 1996). All this evidence supports the idea that pollen could be essential for the Argentine stem weevil egg maturation.

Sexually mature weevils were around 44% of caught weevils over the collection dates, except in winter (i.e., June), when the proportion of sexually mature individuals decreased to 20% (Fig. 7). This is known to occur in the Canterbury region of New Zealand, where diapause takes place from March to August (Goldson and Emberson, 1980; Goldson, 1981b), and that is accompanied by a progressive decline in gonad development of both males and females (Barker *et al.*, 1988). There were no significant third-order interactions between collection date, pollen content, and gonad development. This could be explained by the onset of diapause, which progressive decline in gonad development of the weevils could have influenced the gonad status of the individuals to a greater extent than their pollen consumption.

**Gender and gonad development**

There was a significant second-order interaction between gender and gonad development, whereby the proportions of sexually mature and immature females were significantly different (i.e., 21.8% and 40.5% respectively), whereas the proportions of sexually mature and immature males were almost the same (i.e., 18.6% and 18.9% respectively). This could be
explained by two reasons: different genders contained different amounts of pollen, or the weevils' gonad development of males and females changed differently over the collection dates. However, it was observed that both genders ate similar amount of pollen (Fig. 5), so the more likely explanation is differential responses between genders in gonad condition over collection dates (Fig. 7).

Indeed, most adult weevils enter into diapause in the autumn (Barker et al., 1988b). They exhibit delayed development of their reproductive organs until the following spring (Goldson, 1979 and 1981b; Barker and Pottinger, 1982). The morphological and physiological changes associated with onset of diapause include atrophy of the gonads, cessation of ovulation, resorption of terminal oocytes and cessation of mating (Barker et al., 1988). However, males seemed to be more 'insensitive' to diapause condition than females (Fig. 7). The same trend was observed in the boll weevil (Jones et al., 1992), and the pea weevil (Pesho and van Houten, 1982), where the percentage of reproductive females decreased in winter, whereas the reproductive status of males changed little during these months.

In addition, a change in sex ratio was observed over the collection dates. However, there was a non-significant increase in the sex ratio of field-collected Argentine stem weevils from December (i.e., summer) to June (i.e., winter) and then a non-significant decrease from June to September (i.e., spring, Fig. 9). These results agree with the sex ratio changes observed in the laboratory during experiments carried out with caged Argentine stem weevils (see Chapter 3). Similarly, field-collected boll weevils had a sex ratio (i.e., male/female) of 0.79:1 during early winter, but it increased progressively until late winter months to 1:1 in Massachusetts, USA (Jones et al., 1992). However, the trend found in the present experiment differed from the Argentine stem weevil sex ratio of 1:1 described by Chapman (1984). Moreover, Barker et al. (1988b) found in the North Island of New Zealand, a significantly superior number of males than females, with male/female ratios of 1.26:1. They found this excess of males in peaks of reproductive activity, in October – December and February – April (Barker et al., 1988b).

Changes in sex ratio could be caused by changes in emergence of adult weevils, mortality changes in both or either of the genders, or because different trap methods captured a different proportion of genders. For example, the sex ratios from the literature were based on suction-trapping, wet-sieving and flotation in magnesium sulphate, and sweep-netting sampling.
(Barker et al., 1988b); however, the present experiment used only sweep netting for collection. Further research is needed to identify possible differences in sex ratio captures with different sampling methods. Behavioural (i.e., one gender tending to climb more than the other does) or physical (i.e., body weight or differential strength to cling to a ryegrass leaf) components could vary the sex ratio of the sample.

**Types of pollen**

The types of pollen found in the weevils’ gut were very diverse in terms of the number of families found. However, most of the families identified in the pollen assessment seemed to have been consumed incidentally by the weevils with the consumption of ryegrass leaves; the same phenomenon was observed in pastures in the North Island of New Zealand (G.M. Barker, personal communication, September 2004). Poorly represented in the insects’ gut were tree/shrub families generally, such as Agavaceae 0.1% (e.g., cabbage tree), Betulaceae 0.2% (e.g., birch), Cupressaceae 0.7% (e.g., macrocarpa), Pinaceae 0.1% (e.g., pine), Rosaceae 1.5% (e.g., apple tree), and Salicaceae 0.5% (e.g., willow). If the field-collected weevils had been collected within a paddock instead of along road sides, probably the frequencies of these pollen types would have been even lower. Further research is needed to identify differences in pollen availability and consumption on road sides compared with within-paddock samples.

On the other hand, annual and biannual weeds and pasture species contributed a variable amount of pollen in the insects’ gut, such as Ranunculaceae 0.1% (e.g., buttercup), Plantaginaceae 29.8% (e.g., Plantago major L., P. lanceolata L.), Poaceae 8.7% (e.g., Lolium spp., Poa annua L., Triticum aestivum L., Zea mays L., etc.), and Fabaceae 0.1% (e.g., clover). Probably Fabaceae and Ranunculaceae pollen was also consumed incidentally along with ryegrass leaves as explained before. Nevertheless, further research is needed to identify what pollen frequencies could be considered as incidental or deliberate feeding. Low percentages of pollen could be a product of deliberate consumption, but affected by pollen availability, low pollen requirements by the weevil, low detection techniques, etc. On the other hand, Plantaginaceae and Poaceae pollen frequencies suggest that these types of pollen were deliberately consumed by the weevil. These results partially agree with those found in pastures in the North Island of New Zealand, where weevils fed actively on pollen of P. annua inflorescences (G.M. Barker, personal communication, September 2004). Also, the
Argentine stem weevil evolved feeding on Poaceae plants in its native South America (McNeill et al., 1996). It is possible that the weevil showed certain degree of preference by this type of pollen compared with others. However, further research is needed to identify other plant families which could be consumed by the weevil.

The frequencies of the different pollen types found on the exoskeletons of the weevils were almost always higher than their respective frequencies inside the weevils’ gut. This suggests that even though certain types of pollen were present in the pasture, they could not be consumed by the weevil in high amounts due to low access to the pollen source (i.e., tree/shrub pollen), or feeding was incidental and not due to active feeding on the plant flower/inflorescence (i.e., ryegrass leaves contamination). Nevertheless, Plantaginaceae pollen frequency was lower on the exoskeleton (i.e., 8.6%) compared with the frequency in the weevils’ gut (i.e., 29.8%), which suggests that weevils deliberately fed on inflorescences of this plant family. Similarly, Poaceae pollen was found in higher frequencies in the gut (i.e., 8.7%) than on the exoskeleton (i.e., 0%). Besides deliberate pollen consumption on the inflorescences, it is possible that the weevils groomed their bodies after pollen feeding and consumed the grains obtained by this process.

The proportion of unidentifiable pollen grains was much higher in the gut (i.e., 58.3%) than on the exoskeletons (i.e., 12.3%). This could be explained by pollen degradation due to digestive action in the weevils’ gut as explained before.

Studies of other field-collected weevils, have revealed pollen grains of over a dozen plant families, including: Amaranthaceae, Anarcariaceae, Asteraceae, Brassicaceae, Chenopodaceae, Euphorbiaceae, Fabaceae, Fagaceae, Malvaceae, Poaceae, Polygonaceae and Solanaceae (Rummel et al., 1978; Benedict et al., 1991; Jones et al., 1993; Jones, 1997; Hardee et al., 1999; Cuadrado and Garralla, 2000; Jones and Hardee, 2000; Cuadrado, 2002). The most important plant families in terms of amount of pollen grains consumed were Asteraceae, Poaceae, and Malvaceae, which suggest the importance of Poaceae pollen in the diet of Curculionidae.
Ryegrass particle content
Since the amount of ryegrass consumed by caged Argentine stem weevils decreased to 40% with the addition of pollen to the weevils’ diet (Evans and Barratt, 1995), it was expected that field-collected Argentine stem weevils that fed on pollen would have decreased their ryegrass intake. However, there was no significant interaction between pollen intake and ryegrass content in the gut. This could be due to a lower availability of pollen in the field compared with caged conditions were pollen was easily available in terms of quantity and accessibility. Probably, in the field, pollen was scarce and was eaten sporadically and alternatively to ryegrass. Furthermore, due to the rapid pollen degradation by gastric activity, pollen could be digested much more rapidly in weevils that ate nothing but pollen; probably several weevils that were found with no-pollen and no-ryegrass in their gut had previously fed only on pollen. Further research in needed to elucidate this, a shorter sampling interval could determine more accurately the changes in gut contents of weevils.

There was a significant second-order interaction between gender and ryegrass content in the gut (Fig. 12). Males had a near normal distribution of individuals with no, little or full gut with ryegrass. Conversely, females had a higher proportion of individuals with either little or full gut with ryegrass; only a few individuals had no ryegrass in their gut. This could be explained because females needed to mature eggs in spring or because they have a higher body mass than males (Goldson and Emberson, 1981) and may therefore need to consume more ryegrass.

There was a significant second-order interaction between gonad development and ryegrass content (Fig. 13), whereby males and females with mature gonads generally had their gut full of ryegrass, whereas immature individuals had no or little ryegrass in their gut. This could be explained because individuals that had immature gonads were often parasitised (i.e., 50% parasitism rate based on dissection) and parasitised weevils decrease their ryegrass-feeding rate (Barratt et al., 1996). Alternatively, breaking diapause is characterised by an increase in gonad development and a resumption of feeding activity (Goldson, 1979 and 1981; Barker and Pottinger, 1982). Since gut fullness was assessed in September, when diapause is being broken, probably a higher gonad development associated to a higher ryegrass consumption was a coincidence, that is, weevils could have had an increased gonad development as they broke diapause (i.e., controlled by photoperiod) and a higher ryegrass content as they resumed feeding activity. Further research is needed to explore interactions between gonad
development and ryegrass consumption. Ryegrass content, for instance, should be assessed over all collection dates.

Parasitism
There was a significant and positive correlation between pollen consumption and gonad status of the Argentine stem weevil in the laboratory (Evans and Barratt, 1995) and in the field (i.e., section: Effects of pollen consumption on gonad development, above). Also, the resources carried over from the host vary with the size and nutritional quality of the host (Beckage and Riddiford, 1983; Harvey et al., 1995; Jervis et al., 2001), and parasitoids prefer to oviposit on high quality hosts to satisfy minimum physiological and dietary requirements for larval development and growth (Mackauer and Michaud, 1996; Tagawa et al., 2000). Then, pollen consumption could mean high quality hosts and hence, in theory, a high parasitism rate should be found in those hosts. However, there was non-significant second-order interaction between pollen consumption and parasitism (Fig. 14). This means that M. hyperodae oviposited without making any distinction between both pollen fed and non-pollen fed weevils. It also means that both, parasitised and non-parasitised weevils ate the similar amounts of pollen, independent of their parasitism status.

Although pollen consumption increased gonad development in the weevil, it is not exclusively needed for gonad maturation. Indeed, as discussed previously, ryegrass consumption could have influenced gonad development as well, and ryegrass was more frequently consumed by the weevils, compared with pollen. In theory, M. hyperodae could have used hosts' gonad development as a cue for oviposition, but, due to the low number of individuals that consumed pollen compared with the vast majority that consumed ryegrass, there was no statistical interaction between pollen consumption and parasitism rates. On the other hand, M. hyperodae could have looked for quality cues in the weevils other than gonad development. Other nutritional components of the host could be more important in terms of host choice, such as fat content or muscle development of the host for instance. Further research is needed to investigate what cues are used by M. hyperodae to select its host for oviposition.

A significant second-order interaction was observed between collection dates and parasitism rates. Indeed, the parasitism rate varied widely between collection dates (Fig. 15). The absence of parasitoids in December 2003 agrees with the results of Phillips et al. (1998), who
found extremely low parasitism rates on mid December 1996. The parasitism rates in February and March 2004 of the present experiment also agree with the results of Phillips et al. (1998), who found parasitism rates around 15 to 20% during the same months in 1996. It is important to clarify that not every year is the same in terms of parasitism rates, since environmental conditions, parasitoid and weevil densities vary with time. The absence of parasitoid eggs or larvae in June 2004 was surprising, since the parasitoid overwinters as larvae within the host (Goldson et al., 1993a), hence at least a few parasitoid larvae should have been found in the weevils. Similarly, the high parasitism rate observed in September 2004 was probably explained by a build up of parasitoid populations over the years, since the parasitism rates found by Phillips et al. (1996) were lower for the same month in 1996. These last two collection dates of the present experiment (i.e., June and September) could have also varied compared with the rest of the collection dates because the collection sites varied within the Lincoln area. Considering also that *M. hyperodae* is a parasitoid of low mobility, about 1.5 to 3 km per year (McNeill et al., 2002a), the parasitism rates could vary between locations a few kilometres apart. Further research should sample only one paddock and thus record parasitism rates of a specific location.

**Effects of parasitism on weevil fitness and ryegrass intake**

There were significant second-order interactions both between parasitism and gonad development (Fig. 16), and between parasitism and ryegrass content in the gut (Fig. 17). These results agree with those of Barratt et al. (1996), who reported that parasitised Argentine stem weevils experienced reduced feeding activity and rapid castration (i.e., hence reduction in gonad development) produced by its parasitoid *M. hyperodae*. Indeed, there is a general tendency for solitary koinobiont parasitoids, such as *M. hyperodae*, to reduce host feeding activity. In addition, the advantage of host castration is that it frees for the parasitoid’s immediate use those resources that a host would normally invest in gonads (Quicke, 1997). Also, castration of both male and female weevils was evident during weevil dissection in the present experiment. These findings are extremely important considering that one of the main hypotheses (see chapter 1. General Introduction) of this thesis is based on the theory that the addition of different diets (i.e., pollen) to the host would affect its fitness (i.e., increase in gonad development). A theoretical increase in host fitness would mean more resources available for the developing parasitoid larva and thereby increase the fitness of the parasitoid. Nevertheless, this mechanism worked only with respect to the first part of the theory, where
the addition of pollen to the weevil's diet increased the cumulative number of eggs per female weevil (Evans and Barratt, 1995). However, in the present study, this happened in those weevils that were not exposed to adult parasitoids. Although there was no significant second-order interaction between pollen ingestion and parasitism, which means that parasitised and non-parasitised weevils ate the same amounts of pollen, the presence of parasitoid larvae within the weevils could have caused host castration, which destroyed the pathway of the theory described above.

To study further the hypothesis that the effects of the addition of different diets to the host would affect its fitness and thereby affect the fitness of the parasitoid, a laboratory experiment was designed. This will be detailed in the next chapter.
CHAPTER 3

EFFECTS OF HOST DIET ON COMPONENTS OF FITNESS
OF Microctonus hyperodae LOAN

INTRODUCTION
Evans and Barratt (1995) reported an increased cumulative number of eggs per female of Argentine stem weevil after the addition of pellets of bee-collected pollen to the weevils’ diet (Fig. 18). Several researchers have reported increases in the proportion of female weevils with mature gonads after the addition of pollen to various weevil species’ diet (Pesho and van Houten, 1982; Annis and O’Keeffe, 1984; Jones et al., 1993). Adult wasp fitness is likely to be affected by host nutrition, since resources carried over from immature stages to the adult stage of the parasitoid vary with the size and nutritional quality of the host (Jervis et al., 2001).

Several authors have observed decreases in the proportion of female Argentine stem weevils with mature gonads after the provision of ryegrass with different endophyte strains (Bell and Prestidge, 1991; Popay and Wyatt, 1995; Popay et al., 1995; Barker and Addison, 1996; Popay, 1997). This could mean fewer resources and possibly indirect sublethal effects, caused by the endophyte on the developing parasitoid inside the host, and therefore lower fitness and survival may be expected for parasitoids emerging from these weevils. It is possible endophyte could also influence the fitness of parasitoids by affecting the nutrition of their immature stages.

Floral resources are widely distributed in nature; buckwheat, in particular, has a small, shallow flower where pollen and nectar are easily available to a wide range of insects (Lövei et al., 1993). Stephens et al. (1998) found from 5 to 33 weevils m\(^{-2}\) on flowers of buckwheat patches sown as understorey in apple orchards at Lincoln, New Zealand, but did not specify the weevils’ species. The provision of buckwheat flowers (i.e., nectar) increased the longevity of M. hyperodae more than any other flower species tested by Vattala et al. (2004) and doubled the longevity of the parasitoid compared with water, so is one of the best candidates
for habitat manipulation in conservation biological control of its host, Argentine stem weevil (Vattala et al., 2004). However, given that Argentine stem weevil benefits from pollen (Chapter 2; Evans and Barratt, 1995), it is possible that floral resources targeted at *Melolontha hyperodae* could also increase the fitness of the pest. In conservation biological control, therefore, the relative influences of resource subsidies on pests and their natural enemies (both direct and indirect) must be weighed up, otherwise such subsidies could inadvertently provide greater benefits to the pest than the natural enemy.

![Weevil fecundity](image-url)

**Fig. 18.** Cumulative number of eggs per female Argentine stem weevil with a diet of ryegrass-only, or of ryegrass plus bee-collected pollen (From Evans and Barratt, 1995, used with permission)

Two experiments were conducted to explore the mechanisms involved in this tri-trophic level interaction. The first experiment comprised the addition of ryegrass and/or bee-collected pollen as food sources for the Argentine stem weevil. The second, more elaborate experiment tested additional diets, including buckwheat pollen and ryegrass with endophyte, to further examine these interactions.

This chapter investigates these tri-trophic level interactions on components of parasitoid fitness and survival by manipulating the host diet. The concept of benefiting the third trophic level with ‘resource subsidies’ made available to the second level leads to the suggestion of a new mechanism for enhancing parasitoid fitness, that is ‘indirect’ conservation biocontrol.
MATERIALS AND METHODS

Experiment 1
1. Experiment set up
One thousand Argentine stem weevil adults were collected from the Lincoln area on the 15 August, 2003, using a sweep net. A proportion of these weevils contained immature parasitoids. The weevils were stored at 10°C for approximately 4 days until they were separated into two treatments (control and treatment 1) replicated five times, with 100 weevils per replicate. Then the weevils randomly assigned to the control were fed with endophyte-free ryegrass cv. Tama, simply referred to as ryegrass-only from now on. Two ryegrass bunches were placed in each box and replaced every three days. The weevils in treatment 1 (T1) were fed with ryegrass cv. Tama plus approximately 10 pellets of a pollen mixture (bee-collected pollen pellets; Plate 5) obtained from beehives in Canterbury. Both, ryegrass and bee-collected pollen were replaced every three days.

Both treatments included several moist dental wicks, which were soaked in water every 48 h, so the weevils had access to water. The weevils were held in cages following the Phillips et al. (1996) procedures described in section 3. The parasitoids emerging from weevils in the different treatments and replicates were labelled and stored at -80°C for subsequent measurement of the length of the hind tibia and egg load as described in sections 4 and 5, respectively.

2. Rearing plants for Argentine stem weevil in experiment 1
Ryegrass cv. Tama was planted in a greenhouse a few weeks before the weevils were collected, and was given as seedlings to the weevils. Ryegrass seeds were sown every two weeks to maintain a supply of ryegrass. The seeds were sown in paper pots filled with potting mix.

3. Argentine stem weevil cages
The weevils were held in plastic boxes comprising two chambers, one on top of the other, separated by plastic mesh (holes of 0.5 x 0.5 mm). The plastic mesh was glued to the bottom of the top chamber and prevented adult weevils from falling into the bottom chamber, but
allowed *M. hyperodae* larvae to do so. These boxes were kept at 20°C and 14:10 (L:D) hours photoperiod in controlled temperature cabinets. Newly emerged *M. hyperodae* larvae crawled under tissue paper laid on the floor of the lower chamber to spin their cocoons. The cages were checked for *M. hyperodae* pupae every 24 h.

*Microctonus hyperodae* cocoons were cut out of the paper and placed in a Petri dish, and the number and date of collection of the cocoons was recorded. Petri dishes were kept in the same controlled temperature cabinet at 20°C and 14:10 (L:D) photoperiod. A small cotton bud soaked in water was placed in each Petri dish to maintain high relative humidity. Petri dishes were checked every 24 h, and newly emerged adult parasitoids were transferred to a labelled plastic vial (500 mm height and 15 mm diameter), with a filter paper (10 x 10 mm) soaked in 50:50 honey and water solution and maintained at 20°C and 14:10 (L:D) photoperiod. After 24 h, the parasitoids were stored at -80°C for subsequent measurement of the length of the third tibia and egg load (sections 4 and 5, respectively).

4. **Parasitoid morphological measurement**

The length of a hind tibia was measured following the procedures of Phillips and Baird (2001). The tibia length was measured at 50X magnification using an eyepiece graticule (100 units, 100 subdivisions; Plate 6). To obtain the tibia length in mm, the measurement read in the graticule was multiplied by a factor of 0.02.

5. **Quantifying egg load of the parasitoids**

The eggs were counted using the method of Phillips and Baird (2001), which is described below. The parasitoids obtained from the experiment were either stored at -80°C or dissected less than two hours after they had been killed. To count the eggs, each parasitoid was dissected using two pairs of forceps. The reproductive organs were extracted by carefully pulling the ovipositor posteriorly away from the abdomen. The abdomen was then prised from the metasomal tergum and torn open longitudinally so that any remaining internal organs became exposed. The reproductive organs connected to the ovipositor and the severed abdomen were placed in a watch glass containing a protein stain solution (i.e., 0.1 g nigrosin (BDH) in 100 ml H₂O followed by 3 g trichloroacetic acid).
Plate 5. *Listronotus bonariensis* feeding on bee-collected pollen.

Plate 6. Tibia from the hind leg of *Microctonus hyperodae* ready for measurement.

Plate 7. Dissected abdomen of *Microctonus hyperodae* ready for egg counting.

Plate 8. Pollen in *Listronotus bonariensis* gut.
The watch glass was covered with a glass slip and the parasitoid tissue was left to soak in the stain for 24 h at room temperature. Afterwards, the tissue from each insect was placed on a separate microscope slide with a drop of Hoyers mountant and covered with a glass slip. The stained tissue was then gently agitated by rubbing the upper surface of the glass slip in a circular motion with a pair of fine forceps. This agitation continued, while viewing the stained material under a binocular microscope at 63X magnification, until the parasitoid eggs, which were initially tightly bound together within the ovarioles, were sufficiently separated to permit counting. The eggs were stained dark blue and were readily discriminated from other stained materials by their size (approximately 321 μm long and 24 μm wide) and shape (Plate 7). Eggs were counted at 20X or 64X magnification. After this, the mounted material was sealed by applying nail polish to the joints of the cover slip and the slide, and then labelled recording the treatment, replicate, wasp number, date of mounting and number of eggs.

**Experiment 2**

6. **Experiment set up**

Two thousand and eighty Argentine stem weevil adults were collected from the Lincoln area on the 11 February, 2004, using a sweep net. A proportion of these weevils contained immature parasitoids. The weevils were stored at 10°C for 6 days until they were separated in four treatments (control, treatment 1 to 3, see Table 1) replicated four times, with 130 weevils per replicate. Then the weevils were assigned randomly to the treatments and were fed with the diets listed in Table 1.

<table>
<thead>
<tr>
<th>Major treatment</th>
<th>no pollen</th>
<th>bee-collected pollen</th>
<th>buckwheat flowers</th>
</tr>
</thead>
<tbody>
<tr>
<td>ryegrass</td>
<td>Control</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>ryegrass with endophyte</td>
<td>T3</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Table 1. Combination of treatments of experiment 2
Two ryegrass bunches were placed in each box of control, treatment 1 (T1) and treatment 2 (T2), and replaced every three days. Two ryegrass bunches with endophyte var. Aries containing 94% of the strain ARW of the endophytic fungus *N. lolii*, were placed in each box of the treatment 3 (T3), and replaced every three days. Approximately 10 pellets of a pollen mixture bee-collected in Canterbury was used in the T1. It was replaced every three days. Flowering buckwheat plants cv. Katowase were provided in T2, the apical part of the plant containing the flowers was introduced into each cage through a circular hole (30 mm in diameter) and the hole was sealed with a plastic bag and tape.

All treatments included several moist dental wicks, which were soaked in water every 48 h, so the weevils had access to water. The weevils were held in cages following the Phillips et al. (1996) procedures described in section 3. After the parasitoids had ceased emerging from the weevils, a sample of five weevils per replicate treatment was taken to verify that no parasitoid larvae were still developing in the weevils. At the same time, another sample of 10-15 weevils per replicate treatment was taken to assess the gonad development following the procedures of Goldson and Emberson (1981), and Barker (1989) (section 9). Gut fullness was also assessed (section 10).

7. Methods during and after exposure to parasitoids

The surviving weevils from each of the four replicates were split into two cages and maintained on their respective diets. In one set of cages, 40 weevils per replicate were exposed to one adult parasitoid following the method of Phillips et al. (1996) (section 3). In the second set of cages, the remaining non-exposed weevils were maintained on their respective diets.

The parasitoids which emerged from the exposed weevils were labelled and stored at -80°C for subsequent measurement of the hind tibia length and egg load as described in sections 4 and 5, respectively. One hundred and seventy seven days after initiating the treatments, and 133 days after exposure to parasitoids, the surviving weevils were labelled and stored at -80°C for subsequent assessment of gonad development following the procedures of Goldson and Emberson (1981), and Barker (1989) (section 9). Gut fullness (section 10) and the presence or absence of parasitoid eggs, and whether they were encapsulated, was also assessed.
The non-exposed weevils were maintained in plastic boxes (bottom boxes of Phillips et al., (1996) procedures, section 3) at 20°C and 14:10 L:D photoperiod. One hundred and forty seven days after initiating the diet treatments, and 103 days after the split from the exposed weevils, the surviving non-exposed weevils were labelled and stored at -80°C for subsequent assessment of gonad development following the procedures of Goldson and Emberson (1981) and Barker (1989) (section 9). Gut fullness was also assessed (section 10).

8. **Rearing plants for Argentine stem weevil in experiment 2**

Ryegrass cv. Tama, ryegrass with endophyte var. Aries, and buckwheat cv. Katowase seeds were planted in a greenhouse a few weeks before the weevils were collected. Planting was repeated every two weeks to ensure a continuous supply of food for the weevils.

Ryegrass and ryegrass with endophyte were given as seedlings to the weevils, while buckwheat flowers were grown for their pollen. The buckwheat pollen was provided to the weevils as complete flowers. Seeds of these plants were sown in a greenhouse using paper pots filled with potting mix.

9. **Gonad development in the Argentine stem weevil**

The weevils were dissected in dark purple paraffin wax plaques moulded in 9 cm diameter plastic Petri dishes. Each weevil was secured for dissection by pressing its ventral surface into a small area of wax which had been melted using a hot needle. All work was performed under a microscope at 20X, 40X, or 63X magnification. The elytra were lifted off, and the folded wings were removed. The dorsal surface of the abdomen was opened with forceps and pulled back. This revealed the gut, overlying the gonads. In females, there are two ovaries located dorsolaterally in the abdominal cavity. Each consists of a pair of ovarioles. In reproductively mature females, there are as many as 12 developing oocytes in each ovariole and up to 4 eggs in each calyx, which are enlarged. In males, there is one pair of testes located dorsolaterally in the abdominal cavity, which are divided by sutures into 8-10 wedge-shaped testicular follicles. Seminal vesicles and prostate glands are contiguous with the testes and vary in size according to the level of reproductive activity. Sexually mature males have larger testes, vesicles and glands than do sexually immature males. Weevils with enlarged calyces (and eggs present) or with fully-grown testes, seminal vesicles and prostate glands were considered
as sexually ‘mature’ individuals. The opposite condition was considered as sexually ‘immature’ individuals.

10. Gut fullness of the Argentine stem weevil

10.1. Pollen content
To assess the pollen content on the weevils’ gut, first the weevils were washed with water and detergent for at least one minute, to dislodge pollen grains present on the exoskeleton of the insects (J. Martin, personal communication, November 2003). Then the weevils were dissected as described in the previous section. The gut was removed after severing its anterior and posterior ends and placed on a slide. The method of Irvin et al. (1999) was used to count pollen grains and this is described below.

Each gut was placed on a glass slide in alcohol, and then the gut was teased apart with mounted needles. The gut contents were spread over part of the slide, and then a drop of saffranin (i.e., 0.1% w/v) and a drop of jelly were added, followed by a coverslip. The jelly comprised 7 g gelatine, distilled water to 19 ml, 33 g 82% glycerine, and 1 g phenol crystals. The slides were subsequently scanned under a microscope at 63X magnification (Plate 8). Pollen numbers were estimated on a semi-quantitative scale without detailed counting at the higher pollen categories, into one of seven frequency classes (i.e., 1 = no pollen grains, 2 = 1-10 grains, 3 = 11-100 grains, 4 = 101-1000 grains, 5 = 1001-3000 grains, 6 = 3001-5000 grains, 7 > 5000 grains). To prevent pollen contamination between samples, the forceps and needles were sterilised using alcohol and a Bunsen flame after each dissection (Wratten et al., 1995).

10.2. Ryegrass particle content
The presence or absence of ryegrass in the gut was recorded in both parasitoid-exposed and non-exposed weevils. At the same time as recording pollen grains, the gut fullness was recorded as ‘full gut’, when all the alimentary canal was full of ryegrass particles, ‘little grass’ when ryegrass particles were present only in the foregut or midgut, and ‘no grass’ when no ryegrass particles were observed.

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11. Exposure of weevils to adult parasitoids

Sixteen adult *M. hyperodae* were randomly assigned to exposure cages. Every parasitoid was <48 hours old and was placed with 40 weevils for 24 h, then removed. Filter paper (10 * 10 mm) soaked in 50:50 honey and water solution was placed on the gauze lid of the exposure cage as food for the parasitoid. One bunch of ryegrass was placed in every cage of the treatments that used ryegrass (i.e., T1, T2 and Control, see Table 1), and one bunch of ryegrass with endophyte was placed in every cage of the T3 treatment (i.e., see Table 1). Every exposure cage included one moist dental wick so the weevils and the parasitoid had access to water.

12. Statistical analyses

A generalised mixed model was used to analyse the number of surviving weevils, the number of emerged parasitoids, the time (i.e., days) to 50% parasitoid emergence, and parasitoid tibia length and egg load. However, for the correlation between tibia length and egg load, Pearson correlation coefficients were used. In addition, a Pearson correlation coefficient was used to analyse the correlation between days after weevil exposure and parasitoid egg load.

A log-linear model was used to analyse the statistical significance of the interactions between categorical variables measured in the present experiment (*P* < 0.05). The variables were analysed for second-order interactions between each other, and then for third-order interactions where significant second-order interactions were observed. Also, a Chi-squared ($X^2$) analysis was used to look for further statistical differences between pollen categories.

RESULTS

Experiment 1

*Parasitoid tibia length and egg load*

*Microctonus hyperodae* from the ryegrass-only treatment had a mean tibia length of 0.769 ±0.006 mm (±standard error) (*n* = 46), which was similar to those from the ryegrass plus bee-collected pollen treatment ($F_{1,109} = 1.01, P > 0.05$; Fig. 19), which had a mean tibia length of 0.761 ±0.010 (*n* = 59). The mean egg load of *M. hyperodae* from the ryegrass-only treatment
was 48.4 ±1.7 eggs (n = 44), and was not significantly different (F1,109 = 1.01, \( P > 0.05 \)) from the egg load of the ryegrass plus bee-collected pollen treatment, with a mean of 45.9 ±3.2 eggs (n = 58; Fig. 20).

Overall there was a positive, though non-significant correlation between tibia length and egg load (\( r_p = 0.176, P > 0.05 \); Fig. 21), described by the equation: \( y = 48.598x + 10.089 \). A similar situation was observed in the ryegrass-only (\( r_p = 0.195, P > 0.05 \)) and in ryegrass plus bee-collected pollen treatment (\( r_p = 0.164, P > 0.05 \)).

Fig. 19. Mean ± SE hind tibia length (mm) of *Microctonus hyperodae* from hosts fed either ryegrass-only or ryegrass plus bee-collected pollen. Bars sharing the same letter do not differ at \( P = 0.05 \).
Fig. 20. Mean ± SE egg load of Microctonus hyperodae from hosts fed either ryegrass-only or ryegrass plus bee-collected pollen. Bars sharing the same letter do not differ at $P = 0.05$.

Fig. 21. Correlation between egg load and tibia length (mm) of $M. hyperodae$ parasitoids emerged from hosts fed ryegrass-only or ryegrass plus bee-collected pollen. There was a non-significant difference at $P = 0.05$. 
Experiment 2

**Diet consumption by the weevil**

**Pollen intake**

In the ryegrass plus bee-collected pollen treatment, there was a significant second-order interaction between pollen consumption and parasitoid exposure (i.e., the unparasitised portion of exposed weevils, and non-exposed weevils to adult parasitoids had more pollen in their gut; $F_1 < 0.001, P < 0.05$; Fig. 22). The unparasitised portion of the exposed weevils will be called ‘exposed’ from now on. Forty four percent of non-exposed weevils had pollen in their gut; in contrast, 18% of exposed weevils had pollen in their gut. In addition, there was a third-order interaction between pollen consumption, parasitoid exposure and the field-collected weevils that were used at the beginning of the experiment. At the end of the experiment, weevils exposed and non-exposed to adult parasitoids had significantly higher amounts of pollen in their gut compared with the weevils at the beginning of the experiment ($F_2 < 0.001, P < 0.05$; Fig. 22). There was non-significant difference between the number of weevils which had consumed pollen in exposed weevils fed ryegrass plus bee-collected pollen or ryegrass plus buckwheat pollen ($\chi^2 = 3.818, P = 0.282$). As expected, no weevils from the ryegrass-only, and ryegrass with endophyte treatments had pollen in their gut.

![Fig. 22. Percentage of Argentine stem weevils with pollen in their gut at the beginning of the experiment (A), and at the end of experiment (B), which comprises non-exposed and exposed weevils to adult parasitoids. Bars sharing the same letter do not differ at $P = 0.05$.](image-url)
Ryegrass intake

Exposed and non-exposed weevils consumed similar amounts of ryegrass ($F_1 = 0.879, P > 0.05$). However, there was a significant second-order interaction between ryegrass consumption and treatment ($F_8 < 0.001, P < 0.05$, Fig. 23). For instance, 35% of weevils in the ryegrass-only treatment had their gut full of ryegrass, while this figure was 2% in the ryegrass plus bee-collected pollen treatment, and 21% in ryegrass plus buckwheat pollen and ryegrass with endophyte treatments. There was also a significant second-order interaction between ryegrass and pollen consumption ($F_4 < 0.001, P < 0.05$, Fig. 24); 9.6% of weevils had ryegrass in their gut, and no pollen; in contrast, 1.5% of weevils had ryegrass and pollen in their gut.

Fig. 23. Percentage of Argentine stem weevils with different gut fullness from four treatments. There were significant differences at $P < 0.05$. Analysis was a log-linear model of categorical data. Pair-wise comparisons of dates/categories, etc., were not possible.
Survival and fitness-related data of Argentine stem weevil

Survival

The number of surviving weevils was significantly different ($F_{3,12} = 0.018, P < 0.05$; Fig. 25) among the four treatments. The mean number of weevils was $13.3 \pm 1.1$ ($n = 53$) in the ryegrass-only treatment; $15.8 \pm 2.3$ weevils ($n = 63$) in the ryegrass plus bee-collected pollen treatment; $10.3 \pm 2.4$ weevils ($n = 43$) in the ryegrass plus buckwheat pollen treatment; and $6.5 \pm 0.9$ weevils ($n = 26$) in the ryegrass with endophyte treatment. The ryegrass-only, ryegrass plus bee-collected pollen and ryegrass plus buckwheat pollen treatments were not significantly different ($P > 0.05$). The same pattern was observed between the ryegrass plus buckwheat pollen and ryegrass with endophyte ($P > 0.05$). However, the ryegrass with endophyte treatment was significantly different from ryegrass-only and ryegrass plus bee-collected pollen treatments ($P < 0.05$).
Fig. 25. Mean ± SE number of Argentine stem weevils exposed to adult *Microctonus hyperodae* after the termination of parasitoid emergence, from different treatments. Bars sharing the same letter do not differ at $P = 0.05$.

*Weevil fitness-related data (i.e., gonad development)*

There was a non-significant second-order interaction between gonad development and exposure status of the weevils ($F_1 = 0.183$, $P > 0.05$). However, there was a significant second-order interaction between gonad development and the diet treatments ($F_3 < 0.001$, $P < 0.05$, Fig. 26). The percentage of weevils with mature gonads was 40.5% in the ryegrass-only treatment, 51% in the ryegrass plus bee-collected pollen, 40.5% in the ryegrass plus buckwheat pollen, and 16.5% for the ryegrass with endophyte treatment.

In addition, there was a significant second-order interaction between gonad development and pollen consumption ($F_2 < 0.001$, $P < 0.05$, Fig. 27); of those weevils that had no pollen in their gut, the percentage of sexually mature weevils was 31.3%. However, when weevils had more than 11 pollen grains in their gut, the percentage of sexually mature weevils increased to 97.1%.
Fig. 26. Percentage of Argentine stem weevils with mature gonads at the beginning of the experiment, and from four different treatments at the end of the experiment. There were significant differences at $P < 0.05$. Analysis was a log-linear model of categorical data. Pair-wise comparisons of dates/categories, etc., were not possible.

Fig. 27. Percentage of Argentine stem weevils with mature and immature gonads in relation to different pollen abundance categories. There were significant differences at $P < 0.05$. Analysis was a log-linear model of categorical data. Pair-wise comparisons of dates/categories, etc., were not possible.
Sex ratio

There was a significant second-order interaction between gender and gonad development ($F_1 < 0.001$, $P < 0.05$, Fig. 28). Twenty percent of females were sexually mature over all treatments, but 61% of males were mature over all treatments. However, there was a non-significant interaction between gender and treatments ($F_3 = 0.222$, $P > 0.05$, Fig. 29). In addition, there was a non-significant third-order interaction between gender, sex ratio at the beginning of the experiment, and sex ratio 177 days later ($F_1 = 0.252$, $P > 0.05$, Fig. 29). Furthermore, there was a non-significant second-order interaction between weevil’s gender and pollen consumption ($F_2 = 0.093$, $P > 0.05$).

Fig. 28. Percentage of male and female Argentine stem weevils with mature and immature gonads. There were significant differences at $P < 0.05$. Analysis was a log-linear model of categorical data. Pair-wise comparisons of dates/categories, etc., were not possible.
Fig. 29. Sex ratio of Argentine stem weevil from feeding different treatments over time. There were no significant differences between treatments or days at $P = 0.05$.

**Components of fitness of the parasitoid**

*Body size*

*Microctonus hyperodae* mean tibia length did not vary significantly between treatments ($F_{3,12} = 1.08$, $P > 0.05$; Fig. 30). Parasitoids from the ryegrass-only treatment had the longest tibia at $0.856 \pm 0.010$ mm ($n = 38$). Parasitoids from the ryegrass plus bee-collected pollen treatment had a mean tibia length of $0.848 \pm 0.012$ mm ($n = 33$), and those from the ryegrass plus buckwheat pollen treatment had a mean tibia length of $0.844 \pm 0.012$ mm ($n = 31$). Finally, parasitoids from the ryegrass with endophyte treatment had the shortest tibia length with $0.806 \pm 0.016$ mm ($n = 22$).

![Graph showing sex ratio over time](image)

Fig. 30. Mean ± SE hind tibia length (mm) of *M. hyperodae* parasitoids emerging from different treatments. Bars sharing the same letter do not differ at $P = 0.05$
Egg load
Parasitoids mean egg load did not vary significantly between treatments (F3,12 = 1.08, P > 0.05; Fig. 31). The mean egg load in the ryegrass-only treatment was 46.1 ±1.4 eggs (n = 38), what was almost identical to the mean egg load of the parasitoids from the ryegrass plus buckwheat treatment, with 46.2 ±1.8 eggs (n = 31). Parasitoids from the ryegrass plus bee-collected pollen treatment had the greatest egg load with 48.9 ±2.0 eggs (n = 33); parasitoids from the ryegrass with endophyte treatment had the lowest mean egg load, of 43.0 ±2.4 eggs (n = 22).

Fig. 31. Mean ± SE egg load of *M. hyperodae* parasitoids emerged from different treatments. Bars sharing the same letter do not differ at *P* = 0.05.

Correlation between body size and egg load
Overall there was a positive and significant correlation between tibia length and egg load (*r*ₚ = 0.293, *P* < 0.05; Fig. 32) over the treatments, described by the equation: y = 42.761x + 10.5. The same was observed in the ryegrass-only treatment (*r*ₚ = 0.329, *P* < 0.05). There was also a positive, though non-significant, correlation between tibia length and egg load in the treatments ryegrass plus bee-collected pollen (*r*ₚ = 0.341, *P* > 0.05), ryegrass plus buckwheat pollen (*r*ₚ = 0.095, *P* > 0.05), and ryegrass with endophyte (*r*ₚ = 0.432, *P* > 0.05) treatments. The percentage of variation in egg load accounted for variation in body size, and *P* values are detailed in Table 2.
Fig. 32. Correlation between egg load and tibia length of *Microctonus hyperodae* from different treatments. There were significant correlations in the ryegrass-only treatment, and with all the treatments at $P < 0.05$. Analysis was a log-linear model of categorical data. Pairwise comparisons of dates/categories, etc., were not possible.

Table 2. The relationship between tibia length and egg load for parasitoids emerged from weevils fed with four treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% egg load variation</th>
<th>$P$ - value accounted for tibia length</th>
</tr>
</thead>
<tbody>
<tr>
<td>all treatments</td>
<td>8.6</td>
<td>0.001</td>
</tr>
<tr>
<td>ryegrass-only</td>
<td>10.8</td>
<td>0.044</td>
</tr>
<tr>
<td>ryegrass plus bee-collected pollen</td>
<td>11.6</td>
<td>0.053</td>
</tr>
<tr>
<td>ryegrass plus buckwheat pollen</td>
<td>0.9</td>
<td>0.608</td>
</tr>
<tr>
<td>ryegrass with endophyte</td>
<td>18.6</td>
<td>0.051</td>
</tr>
</tbody>
</table>

**Development time**

Overall there was a positive, though non-significant, correlation between days after weevil exposure to adult parasitoids, and the egg load of the parasitoids emerging from weevils fed different treatments ($r_p = 0.039, P > 0.05$; Fig. 33), described by the equation: $y = 0.0193x + 44.786$. This correlation was negative and non-significant in the ryegrass-only treatment ($r_p = -0.132, P > 0.05$) and ryegrass with endophyte ($r_p = -0.254, P > 0.05$). However, the correlation was positive and non-significant in the ryegrass plus bee-collected pollen ($r_p = 0.175, P > 0.05$) and ryegrass plus buckwheat pollen ($r_p = 0.092, P > 0.05$).
There were non-significant differences in the time to 50% emergence of the parasitoids over the treatments ($F_{3,11} = 0.46, P > 0.05$; Fig 34). The lowest mean time to 50% of parasitoid emergence was $72.0 \pm 9.5$ days ($n = 38$) in the ryegrass-only treatment. This was similar to the mean of $73.0 \pm 7.9$ days ($n = 22$) in the ryegrass with endophyte treatment. The time taken 50% of parasitoid emergence was highest in the ryegrass plus buckwheat pollen, at $80.5 \pm 7.3$ days ($n = 31$), followed by the ryegrass plus bee-collected pollen treatment, with $80.3 \pm 5.9$ days ($n = 33$).

![Graph showing mean days to 50% parasitoid emergence across different treatments.](image)

**Fig. 33.** Correlation between egg load and days after *Microctonus hyperodae* emergence. There were no significant differences at $P = 0.05$.

![Bar chart showing mean days to 50% parasitoid emergence across different treatments.](image)

**Fig. 34.** Mean ± SE days to 50% of parasitoid emergence from weevils feeding on different treatments. Bars sharing the same letter do not differ at $P = 0.05$
**Number of adult parasitoids emerged**

Overall, there were non-significant differences in the number of adult parasitoids emerging from the treatments ($F_{3,12} = 0.69$, $P > 0.05$; Fig 35). The highest mean number of parasitoids was $9.5 \pm 2.5$ (n = 38) in the ryegrass-only treatment. This was followed by $8.3 \pm 0.8$ (n = 33) in the ryegrass plus bee-collected pollen treatment. Then followed by $7.8 \pm 2.4$ (n = 31) in the ryegrass plus buckwheat pollen treatment, and the lowest mean was $5.5 \pm 1.8$ (n = 22) in the ryegrass with endophyte treatment.

![Bar chart showing number of adult parasitoids](image)

Fig. 35. Mean ± SE number of adult *Microctonus hyperodae* emerged from different treatments. Bars sharing the same letter do not differ at $P = 0.05$

**DISCUSSION**

It was hypothesised that the provision of different diets to the host would affect its fitness and thereby affect the fitness of the parasitoid (Fig. 20). After providing different diets to hosts, the following types of effects are possible: 1) affects on both host and parasitoid; 2) affects only on one species and not on the other; 3) affects neither host or parasitoid. These effects could be positive or negative depending on whether they increase or decrease fitness. These combinations of positive, negative or no effects produced in each animal, are used to frame this discussion.
Fig. 36. Hypotheses about how the diet manipulation of the host could affect its parasitoid. Possible effects on number of individuals and/or their fitness are represented by: “↑” = positive effect, “↓” = negative effect

Experiment 1

Parasitoid body size and egg load

It was hypothesised that bee-collected pollen would increase the fitness of the host, perhaps via an increase in gonad development, and thereby enhance fitness of the parasitoid. However, the tibia length and egg load of parasitoids from the ryegrass-only and ryegrass plus bee-collected pollen treatments did not differ significantly (Fig. 19 and 20). Moreover, the correlation between tibia length and egg load was non-significant in experiment 1 (Fig. 21). Phillips and Baird (2001) found that *M. hyperodae* from east of the Andes mountains showed no significant correlation between egg load and hind tibia length, yet parasitoids from Chile showed a significant, though weak, positive correlation. The data obtained from experiment 1 are, therefore, consistent with the occurrence of a high frequency of eastern *M. hyperodae* in the present study. Indeed, eastern *M. hyperodae* has been shown to be the more successful of the two strains throughout New Zealand (Phillips et al., 1997; Phillips et al., 2004). Although
the addition of bee-collected pollen to the host diet had no significant effect on the parasitoids' body size, egg load, or the relationship between these two variables, there are some factors that could have partly obscured a positive correlation between parasitoid fitness and pollen consumption by the weevils, and these are discussed below.

Experimental set up
Since some of the weevils used for the experiment 1 had been parasitised in the field, the parasitoids which emerged had completed part of their development before to the beginning of the experiment, and hence before to the provision of different diets to the weevils. As a result, the potentially better nutrition from the ryegrass plus bee-collected pollen treatment may have been provided too late to detect any difference between treatments. For example, when larvae of *Drosophila melanogaster* L. (Diptera: Drosophilidae) were fed yeast immediately after parasitisation, they were able to encapsulate the eggs/larvae of its parasitoid, *Leptopilina boulardi* (Barbotin, Carton & Kelner-Pillault). However, when *D. melanogaster* larvae were fed yeast 24 h after parasitisation, the diet had no effect on the immune response (Vass and Nappi, 1998).

To account for this possibility, experiment 2 provided the diets to unparasitised weevils before they were exposed to adult parasitoids in the laboratory. In this way, the weevils could experience diet-related effects before to parasitism, and hence the parasitoid larva completed its entire development within a host subjected to one dietary regime.

Weevil physiology
1. Weevil age
Since the weevils used for the experiment 1 were captured in August, it is likely that they belonged to the second generation from the previous summer (Barker et al., 1988b). Mortality of the overwintering generation reaches a peak in early December (Phillips et al., 1998). Therefore, the weevils captured in August that were used in experiment 1 were old weevils. This could have decreased their response to the pollen diet and obscured any changes in parasitoid fitness. For example, Sequeira and Mackauer (1992) observed that old hosts produced parasitoids with decreased fitness. Furthermore, Tagawa et al. (2000) demonstrated that certain components of fitness of the hyperparasitoid, *Eurytoma goidanichi* Boucek, were reduced when they emerged from old hosts compared with those from young hosts, and they
argued that old hosts provided low quality resources. In experiment 2, younger weevils belonging to the early second generation (i.e., captured in February) were used.

2. Immune system

It is possible weevils fed ryegrass plus bee-collected pollen increased their immune response and were able to encapsulate more parasitoid eggs or larvae than the rest of the weevils fed ryegrass-only. This mechanism has been demonstrated in the vinegar fly, *D. melanogaster*. When the fly larvae were fed with yeast immediately after parasitism by the wasp *L. bouardi*, the host larvae were able to encapsulate a significantly higher percentage of the parasitoid’s eggs than did hosts deprived of yeast (Vass and Nappi, 1998). However, no weevil dissection was performed in experiment 1 to assess the number of eggs/larvae encapsulated within hosts, but such dissections were performed in experiment 2.

Experiment 2

*Diet consumption by the weevil*

*Pollen intake*

The Argentine stem weevils used for the present experiment had pollen in their gut before collection (i.e., pollen consumed in the field), and after collection (i.e., pollen consumed in the laboratory). However, in the laboratory, pollen consumption changed with parasitoid exposure. Weevils exposed to adult parasitoids ate significantly less pollen than did the non-exposed weevils (Fig. 22). Similarly Barratt *et al.* (1996) and Gerard (2000) observed that the unparasitised portion of exposed weevils to adult parasitoids exhibited decreased feeding activity, compared with non-exposed weevils. Parasitoid presence had a detrimental effect on pollen consumption even in the unparasitised portion of weevils exposed to adult parasitoids, because physical disturbance of the weevils by the parasitoids resulted in a reduction of weevil feeding activity during the period of exposure (Barratt *et al.*, 1996; Gerard, 2000).

Even though parasitoids reduced weevil feeding, exposed and non-exposed weevils that were provided pollen diets in the laboratory had significantly higher amounts of pollen in their gut compared with the weevils in the beginning of the experiment (Fig. 22). This suggests pollen availability may limit pollen consumption by Argentine stem weevil in the field (Chapter 2).
Although the number of exposed weevils taking pollen did not differ significantly between the ryegrass plus bee-collected pollen and ryegrass plus buckwheat pollen, the numbers of pollen grains consumed were different. Seven percent of the exposed weevils fed ryegrass plus buckwheat pollen had from 1 to 10 grains of pollen in their gut, and none had more than 10 pollen grains in their gut. In contrast, of the exposed weevils fed ryegrass plus bee-collected pollen, 7% had between 1 to 10 pollen grains in their gut, 12% had more than 10 pollen grains in their gut, and 2% of the weevils had up to 3000 pollen grains in their gut. The lower number of pollen grains consumed in the ryegrass plus buckwheat pollen treatment could be explained by more difficult access to buckwheat pollen in this experiment. In the ryegrass plus buckwheat treatment, weevils had to climb the buckwheat stems to reach the flowers and eat the pollen, but in the ryegrass plus bee-collected pollen treatment, bee-collected pollen was more readily accessible because it was provided as pollen-pellets in the bottom of the cage. However, incidental pollen consumption via ryegrass leaves contaminated with buckwheat pollen could also explain this observation. Buckwheat flowers were placed on top or beside the ryegrass, so pollen from buckwheat anthers could have been shed on top of the ryegrass leaves. In contrast, bee-collected pollen was placed in the bottom of the cage, so contamination of ryegrass leaves by gravity was unlikely.

Ryegrass intake

Several researchers have reported that endophyte reduces feeding activity and damage caused by Argentine stem weevil (Bell and Prestidge, 1991; Popay and Wyatt, 1995; Popay et al., 1995; Barker and Addison, 1996; Popay, 1997) and other insect pests (Bultman and Bell, 2003; Bultman et al., 2004). In the present study, the ryegrass intake of the weevils fed ryegrass with endophyte was lower compared with weevils fed ryegrass-only (Fig. 23).

The weevils’ ryegrass intake in the ryegrass plus bee-collected pollen treatment was significantly lower compared with the ryegrass-only treatment (Fig. 23). This agrees with the results of Evans and Barrat (1995), who observed that the amount of ryegrass consumed by weevils provided with pollen and ryegrass was approximately 45% lower than those provided with ryegrass alone. It also agrees with the result that there was a significant and negative second-order interaction between ryegrass and pollen consumption (Fig. 24). Weevils fed ryegrass plus buckwheat also had a lower ryegrass intake compared with weevils fed ryegrass-only, which could be explained the same way. Although weevils fed ryegrass plus
buckwheat had a higher ryegrass intake compared with those fed ryegrass plus bee-collected pollen (Fig. 23), this could be explained due to the lower pollen consumption of weevils fed ryegrass plus buckwheat compared with weevils fed ryegrass plus bee-collected pollen.

**Survival and fitness of Argentine stem weevil**

**Survival**

Weevil survival was influenced by the rates of parasitism that occurred in each treatment, and may also have been influenced by weevil diet. In these experiments, there was no significant difference between treatments in parasitism rates (see section ‘Number of adult parasitoids emerged’ below). However, the survival of weevils fed ryegrass with endophyte was significantly lower than weevils from ryegrass-only and ryegrass plus bee-collected pollen treatments (Fig. 25). Barker and Addison (1996) also reported a decrease in weevil survival when fed ryegrass with endophyte compared with weevils fed ryegrass-only. This decrease in survival could have been produced by starvation of the weevils due to deterrence by the endophyte, or a toxic effect of the endophyte after consumption. In contrast, the survival of weevils fed ryegrass-only, ryegrass plus bee-collected pollen and ryegrass plus buckwheat pollen was not significantly different (Fig. 25). These results agree with those found by Evans and Barratt (1995) who observed no difference in survival of weevils fed ryegrass-only or ryegrass plus bee-collected pollen.

**Weevil fitness-related data (i.e., gonad development)**

Non-exposed weevils and the unparasitised portion of exposed weevils exhibited similar proportions of individuals with mature gonads. These results were different from those of Barratt *et al.* (1996), who observed that the fecundity of Argentine stem weevil was progressively reduced as exposure to adult parasitoids increased. However, these researchers assessed the gonad development of the weevils based on counts of eggs laid per female, not on dissections of both genders as was done in the present experiment. It is possible in the experiment of Barratt *et al.* (1996) that adult parasitoid presence could have affected the oviposition behaviour of the female weevils, but not their gonad development.

It was hypothesised that the addition of pollen to the weevils’ diet would increase their gonad development. Conversely, the addition of ryegrass with endophyte would decrease the gonad
development. Indeed, weevils fed ryegrass with endophyte experienced a drastic decrease in gonad development (Fig. 26) which could be explained either by toxic effects of the endophyte on the weevils, or by starvation. Several authors have also observed that Argentine stem weevil had a lower oviposition rate (Bell and Prestidge, 1991; Popay and Wyatt, 1995; Popay et al., 1995; Popay, 1997) and decreased gonad development (i.e., oocyte resorption) (Barker and Addison, 1996) when fed ryegrass with endophyte compared with ryegrass-only. Oosorption occurs at a lower level in female weevils fed non-endophyte ryegrass (Barker et al., 1988b). However, the ryegrass plus buckwheat pollen treatment did not increase gonad development. Weevils that were fed ryegrass plus buckwheat pollen tended to have slightly lower gonad development than weevils fed ryegrass-only (Fig 26). Although consumption of just 1-10 pollen grains increased weevil gonad development (i.e., considering bee-collected and buckwheat pollen altogether. Fig. 27), buckwheat pollen alone did not increase gonad development (Fig. 26). This could be explained by a lower nutritional quality of buckwheat pollen. In pea weevil, for example, the proportion of females developing eggs appeared to be due to the low nutritional quality of pea pollen (Annis and O’Keeffe, 1984). In contrast, the higher gonad development of weevils fed ryegrass plus bee-collected pollen (Fig. 26) could be due to better nutritional quality of bee-collected pollen. Pesho and van Houten (1982) demonstrated that oocyte growth of female pea weevils was enhanced by the addition of pea pollen to the weevils’ diet of alfalfa, *Medicago sativa* L., leaves. Similarly, ovariole width in boll weevils was promoted after pollen intake (Jones et al., 1993).

It has been demonstrated that the addition of pollen to the weevils’ diet increased their gonad development compared with weevils fed ryegrass-only (Evans and Barratt, 1995). However, over all treatments, gonad development declined between the start and end of the experiment (Fig. 26). These results differ from those of Evans and Barratt (1995), who observed an increase in the cumulative number of eggs per female weevil over time in those weevils fed ryegrass plus bee-collected pollen, compared with weevils fed ryegrass-only. One or more factors could have produced this difference including the onset of diapause in the weevils, weevil density, or sex ratio changes.

1. **Diapause effect**

The lights of the laboratory that would produce a 14:10 L:D photoperiod (i.e., weevil critical day-length 13.3:10.7 L:D; Goldson et al., 1993a) were turned off by mistake from when the
experiment was set up until 68 days later when they were turned on. The weevils therefore probably entered diapause soon after the start of the experiment. In addition, some weevils could already have been in diapause before the experiment began since these weevils belonged to the second generation, which enters directly into diapause in autumn (Barker et al., 1988), independently of temperature (Goldson and Emberson, 1980). In addition, second-generation adult weevils do not become reproductively mature before winter, probably to preserve resources until the following spring (Goldson, 1979 and 1981b; Barker and Pottinger, 1982). The morphological and physiological changes associated with onset of diapause include atrophy of the gonad, cessation of ovulation, resorption of terminal oocytes and cessation of mating (Barker et al., 1988b). The onset of diapause is likely to have reduced the response of gonad development to pollen.

2. Weevil density
It is possible that the weevil density used in the present experiment was too high (140 weevils m\(^{-2}\)). Barker et al. (1989) reported that weevil densities above 75 adults m\(^{-2}\) produced rapid decline in ovarian condition and oviposition effort per female weevil. This was also suggested by McNeill et al. (1998) to explain 40% of non-reproductive female weevils in their experiment. Similarly, Goldson (1979) discovered that caging fully reproductive weevils led to a dramatic decline in the proportion of females with eggs from about 90 to 20% in only 10 days. However, Evans and Barratt (1995) used 178 field-collected weevils m\(^{-2}\) in their experiment, and found an increase in the cumulative number of eggs per female in weevils fed ryegrass-only over time. Further research is needed to elucidate interactions between weevil density and gonad development.

3. Sex ratio
There was a significant second-order interaction between gender and gonad development (Fig. 28). Female Argentine stem weevils had a significantly higher proportion of sexually immature individuals compared with male weevils (Fig. 28). This could be explained by the onset of diapause, which affects mainly female weevils' gonad development (Barker et al., 1988b). A similar situation was observed in the pea weevil, where males were sexually mature and females were sexually immature when leaving diapause (Pesho and van Houten, 1982). A decrease in male Argentine stem weevil population over time could have influenced the decrease in gonad development observed in the present experiment. However, the sex
ratio did not change significantly over time, or between treatments. Furthermore, pollen had no effect on male or female weevil survival, since the sex ratios in the treatments were not significantly different (Fig. 29). This agrees with Evans and Barratt (1995) who observed no significant increase in survival of female weevils fed ryegrass plus bee-collected pollen compared to female weevils fed ryegrass-only.

**Fitness of the parasitoid**

**Body size**

The length of the hind tibia of parasitoids that emerged from hosts fed different diets did not significantly differ between treatments (Fig 30). It is possible that the general decrease in gonad development of the weevils over time (independent of treatment) could have decreased the resources made available for the developing parasitoid. This could have obscured any potential increase in parasitoid size in the present experiment. However, the length of the hind tibia of parasitoids that emerged from the ryegrass with endophyte treatment did not differ from those emerging in the other treatments, even though weevils fed ryegrass with endophyte had a much lower proportion of weevils with mature gonads. These results are consistent with research on other plant resistance strategies such as transgenics. For example, the parasitoid wasps *Eulophus pennicornis* (Nees) and *Aphelinus abdominalis* (Dalman) emerging from hosts fed transgenic (i.e., GNA gene) plants of tomato and potato respectively, did not differ in body size compared with wasps emerged from hosts fed the non-transgenic version of the crops (Bell *et al*., 1999; Couty *et al*., 2001).

Also, based on the fact that parasitised weevils are rapidly sterilised after parasitism (Barratt *et al*., 1996), it is possible that *M. hyperodae*’s body size was based on resources other than weevils’ gonads (e.g., flight muscles, body fat, etc.). Hymenopteran parasitoids require approximately 30 chemicals to fulfil their nutritional requirements, including proteins and/or amino-acids, B-vitamin complex, energy (i.e., carbohydrates or lipids), fat soluble vitamins, cholesterol, and polyunsaturated fatty acids (Thompson and Hagen, 1999). The parasitoid food is of animal origin, thus being generally high in protein content and low in carbohydrate and fat (Thompson and Hagen, 1999). Theoretically, other compounds such as fatty acids could be limiting factors for *M. hyperodae* larvae. Indeed, Argentine stem weevils in the field have 16% of dry weight in fat during winter, which decreases to 8% with the beginning of egg maturation (Goldson, 1981b). This decrease in fat could play an important role in the nutrition
of the parasitoid. Further research is needed to identify nutritional requirements associated to the larval development of *M. hyperodae*. Protein, lipid and carbohydrate foods could be tested as possible candidates to increase the parasitoid’s body size.

It is possible that other components of fitness of *M. hyperodae* were affected by the nutrition of its host (e.g., longevity or searching efficiency). Further research is needed to identify other components of fitness of the parasitoid that could be increased with the addition of pollen to the host diet.

Features of parasitoid development such as teratocytes and the storage of nutrients in the parasitoid egg may help to compensate for hosts of low nutritional quality and thus reduce any effects of host diet on parasitoid fitness. Teratocytes are cells derived from the embryonic membrane of parasitoid eggs, which are released into the host haemocoel at the time of egg hatching. They appear to play a role in parasitoid nutrition (Thompson and Hagen, 1999). Sluss (1968) demonstrated that teratocytes of the parasitoid wasp *Perilitus coccinellae* (Schrank) (Hymenoptera: Braconidae) increased in volume several fold in the coccinellid host and were subsequently eaten by the developing parasitoid larvae. Furthermore, stored nutrients within the parasitoid egg can support limited development of the parasitoid larva (Thompson and Hagen, 1999). For example, due to nutrients stored within the parasitoid egg, partial larval development of the parasitoids *Itopectis conquistor* (Say) (Yazgan, 1972) and *Exeristes roborator* (Fabricius) (Thompson, 1981) was achieved with diets lacking various essential amino-acids and B-complex vitamins. During the dissection of Argentine stem weevils, teratocytes were frequently observed in parasitised individuals. Further research is needed to understand the mechanisms which maintain parasitoid fitness in variable quality hosts.

**Egg load**

Egg loads of parasitoids that emerged from hosts fed different diets did not differ significantly between treatments (Fig. 31). It is possible that *M. hyperodae*’s egg load was based on other resources (e.g., flight muscles or body fat) carried from the host as discussed previously in the body size section. Further research is needed to explore other nutritional factors associated with the weevil that could affect parasitoid egg load.
It seems likely that diapausing weevils provided parasitoids with similar nutrition to non-diapausing weevils since the average egg load of the ryegrass-only treatment of 46.1 eggs recorded in the present experiment corresponded well to the mean egg load of 47 eggs recorded from non-diapausing weevils fed ryegrass-only by Phillips and Baird (2001).

It was expected that *M. hyperodae* egg loads would be lower in the ryegrass with endophyte treatment, as other authors have reported for other insect resistant crops. For instance, adults of the endoparasitoid *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) which emerged from hosts fed with transgenic cotton plants expressing the gene *Cry1A(c)* had lower egg loads than those which emerged from hosts fed the non-transgenic version (Baur and Boethel, 2003). However, *M. hyperodae* egg load did not decrease with the addition of endophyte to the weevils’ diet. Similar results were reported for the parasitoid wasps (*E. pennicornis* and *A. abdominalis*) that emerged from hosts fed transgenic plants of tomato and potato, respectively, expressing the GNA gene. These parasitoids had similar egg loads to parasitoids that emerged from hosts fed with the non-transgenic version of the crops (Bell *et al.*, 1999; Couty *et al.*, 2001). This suggests that biological control and plant resistance may often work in a complementary, or even synergistic, way in integrated pest management programmes (van Emden, 1990).

*Correlation between body size and egg load*

The carry-over of resources from the host is often positively correlated with wasp body size (Harvey *et al.*, 1995; Jervis *et al.*, 2001), and adult body size is generally correlated with egg load at emergence (Jervis *et al.*, 2001). However, *M. hyperodae* is an unusual case where some geographic populations (e.g., those from Chile and from San Carlos de Bariloche in the Argentinean Andes) exhibit relatively strong correlations between body size and egg load, while other geographic populations from east of the Andes do not (Phillips and Baird, 2001). In experiment 2, the correlation between tibia length and egg load of the parasitoid over all treatments was positive and significant, though weak (Table 2, Fig. 32). A significant, though weak, correlation was also found in the ryegrass-only treatment. However, in the ryegrass plus bee-collected pollen, ryegrass plus buckwheat pollen, and ryegrass with endophyte treatments, the result was nearly significant.
These results differ from experiment 1, where the correlation was non-significant. It is possible that a mixture of parasitoid lineages from the east and west of the Andes were present in the experiment 2 (Phillips and Baird, 1996; Phillips et al. 2004; Iline and Phillips, 2004). Indeed, the percentage of egg load variation accounted for by the tibia length of parasitoids that emerged from weevils fed ryegrass-only was 10.8% (Table 2), which corresponded well with the 10.6% obtained by Phillips and Baird (2001) using a mixture of parasitoid lineages that had also emerged from weevils fed ryegrass-only. Furthermore, the percentage of egg load variation accounted for the tibia length, considering all the treatments, was generally low (i.e., 8.6%; Table 2) and this corresponded well with the results obtained by Phillips and Baird (2001) for the parasitoid population coming from east of the Andes in South America (i.e., 8.5% on average for parasitoids from Argentina, Uruguay, and Brazil). Based on these results, it is probable that the parasitoids obtained in the present experiment were mainly from populations derived from material from east of the Andes. Further research should include parasitoid morphological identification, DNA and enzyme to determine its South American origin.

Developmental time
The onset of weevil diapause probably affected the development time of *M. hyperodae* since it enters photoperiodically-induced diapause as a first instar larva (Goldson and McNeill, 1992), with a critical photoperiod of 12.3: 11.7 L:D, irrespective of the physiological condition of the host (Goldson *et al.*, 1993a). Diapause would explain why the emergence of parasitoid prepupae began 40 days after the exposure of the weevils to adult parasitoids, instead of after approximately 14 days at 20°C as would normally be expected (Loan and Holdaway, 1961; C.B. Phillips, personal communication, July 2004), and ceased 129 days after exposure.

Barker and Addison (1996) demonstrated that the development of *M. hyperodae* larvae was retarded when they developed inside Argentine stem weevils fed ryegrass with endophyte compared with those developing inside weevils fed endophyte-free ryegrass. Similar results were reported by Bultman *et al.* (2003), who found that Argentine stem weevils fed some strains of endophyte affected the development rate of *M. hyperodae*. Similarly, the larval development rate of the braconid endoparasitoid *C. marginiventris* was significantly retarded when developing inside hosts fed transgenic cotton expressing the Cry1A(c) gene compared
with parasitoids that had emerged from hosts fed the non-transgenic version of the cultivar (Baur and Boethel, 2003). However, there was no significant difference in parasitoid development rates between treatments in the current experiment. This suggests diapause, rather than toxic effects from endophytes, is the most likely explanation for the observed protracted development of *M. hyperodae* in the current experiment. It is notable that the endophyte strain ARW (i.e., the same used in the present experiment), had no retardant effects on the development of *M. hyperodae* (Bultman et al., 2003).

The long development time of the parasitoid larva could have influenced parasitoid egg load. For example, parasitoids that spent more time in the host may have had more time to accumulate resources. However, this did not appear to be so because there was only a weak non-significant correlation between days after exposure and parasitoid egg load (Fig. 33), and the time to 50% of parasitoid emergence was similar for all the treatments (Fig. 34).

The developmental delay caused by diapause probably did not obscure any treatment effects since the larval parasitoids and their hosts probably responded to the lights being turned on simultaneously across treatments. Such phenological synchrony between parasitoids and their hosts has been widely reported. For example, larvae of *Microctonus aethiops* (Nees) (Hymenoptera: Braconidae) developed quickly in alfalfa weevils, *Hypera postica* (Gyllenhal), that were sexually mature, but entered diapause in sexually immature weevils (Day 1971). This dormant period served to synchronise the parasite’s life cycle with that of its host (Day, 1971).

**Number of adult parasitoids emerged**

In the present experiment, the number of adult parasitoids that emerged from the different treatments did not significantly differ (Fig. 35). These results agree with those of Bultman et al. (2003) who did not find significant differences in the number of parasitoids that emerged from laboratory reared weevils fed ryegrass-only or ryegrass with ARW endophyte. However, in the present experiment the sample variance was very high, probably due to the small number of wasps that emerged from every replicate. Further research would need to use more weevils, to increase parasitoid numbers and lower the variance.
There was a trend where the number of parasitoids that emerged from the ryegrass plus bee-collected pollen and ryegrass plus buckwheat pollen treatments were lower than the ryegrass-only treatment. It is possible that the provision of pollen increased the weevils' immune response so they could encapsulate more parasitoid eggs or larvae than weevils fed other diets. However, based on weevil dissections performed after parasitoid exposure, only one out of 65 weevils in the ryegrass plus bee-collected pollen treatment had an encapsulated parasitoid egg, and no encapsulated parasitoids were found in the other treatments. Enhanced weevil immune responses therefore cannot explain the trend for reduced numbers of adult parasitoids from the ryegrass plus bee-collected pollen treatment.

There was also a trend where the number of parasitoids that emerged from the ryegrass with endophyte treatment was lower than the ryegrass-only treatment. It is possible that secondary poisoning of M. hyperodae larvae occurred. Plant resistance relying on toxic allelochemicals may affect parasitoids, and thereby indirectly change parasitoid-host interactions (Bultman et al. 1997). These chemicals can produce toxicity to parasitoids within hosts or otherwise reduce parasitoid emergence from them (van Emden, 1990). In fact, artificial diets containing the diterpenes lolitrem B and α-paxitriol of endophyte origin, reduced the survival of M. hyperodae larvae within hosts at concentrations of 2 μg g⁻¹ (Barker and Addison, 1996). Indeed, Lolitrem B was present in the AR W endophyte strain used for the present experiment. Further research should include dissection of alive parasitised weevils at regular time intervals to elucidate the within-host mortality of M. hyperodae larvae.

It is also possible that endophyte reduced the parasitism rate. For example, Barker and Addison (1996, 1997) reported a significantly lower parasitism rate by M. hyperodae on weevils fed on ryegrass with endophyte (i.e., 66.3% parasitism), than in weevils fed on ryegrass-only (i.e., 78.2% parasitism). Based on weevil collection, Goldson et al. (2000) observed 34.1% parasitism in ryegrass with endophyte and 63.6% parasitism on ryegrass-only. There was a significant inverse relationship between parasitism rate and peramine levels in the field. They concluded that adult M. hyperodae parasitoids probably were less successful attacking hosts on high endophyte plots in the field, because of the reduced feeding activity of the weevils (Goldson et al., 2000; Phillips, 2002). Indeed, several authors have reported that Argentine stem weevil had a lower feeding activity in ryegrass with endophyte, compared with those feeding on ryegrass-only (Bell and Prestidge, 1991; Popay and Wyatt, 1995; Popay et al., 1995; Popay, 1997). Also, adult weevils were more susceptible to parasitism when they
were moving, particularly when feeding (Phillips, 2002). Moreover, Gerard (2000) observed that about 90% of the adult weevils on ryegrass with endophyte were in positions unfavourable (i.e., crouching and off plant) for parasitoid oviposition and less time feeding, compared with weevils on ryegrass-only. Based on these ideas, it is possible that, in the present experiment, the weevils fed ryegrass with endophyte experienced a lower parasitism rate compared with the rest of the treatments. However, due to the low number of parasitoids that emerged, the variability was too high and the differences were not significant. However, Exner and Vidal (1996) found that endophyte in tomatoes (Lycopersicum esculentum L.) had no effect on the attack rate of Encarsia formosa Gahan (Hymenoptera: Trichogrammatidae) on second instar larvae of the whitefly Trialeurodes vaporariorum (Westwood) (Homoptera: Aleyrodidae).

It is possible that diets with deterrent effects such as endophytes can also cause host starvation (Bultman et al., 1997) and could also prevent parasitoids from emerging. For example, the parasitoid Cotesia congregata Say (Hymenoptera: Braconidae) failed to emerge if its host larva, Manduca sexta (L.) (Lepidoptera: Sphingidae), was starved (Beckage and Riddiford, 1983). However, as discussed in the ryegrass intake section, starvation was unlikely to have occurred in the present experiment. Further research is needed to understand the interactions between M. hyperodae and endophytes.
CHAPTER 4

GENERAL DISCUSSION

THE HOST’S MARK

“.........far from being a purely passive victim, obliterated without a trace, the host is often able to impress its mark ...... upon the insect parasitoid that destroys it” (Salt, 1941). In the present work, the number of parasitoids emerged and their fitness as a product of different diets given to the host has been the “mark” investigated. The literature in this respect is rather scarce concerning those “impressed marks” left by the host on its parasite (Salt, 1941; Beckage and Riddiford, 1983; Harvey et al., 1995; Bernal et al., 1999; Tagawa et al., 2000; Honda and Luck, 2001). Furthermore, as will be discussed below, even plants or parts of them such as pollen (i.e., the first trophic level) can shape this mark and influence the interactions of the trophic levels that follow.

Pollen has evolved for its role in sexual reproduction in plants, but only a small fraction arrives on another flower, while the remaining pollen has other ecological values (van Rijn et al., 2002). Pollen can be utilised by several groups of predatory (e.g., Wratten et al., 1995), parasitic (Zhang et al., 2004) and herbivorous (e.g., Jones et al., 1993) arthropods. Due to the high nutritional value of pollen (Thompson and Hagen, 1999), and its importance in egg maturation in some species of predatory (e.g., adult syrphid flies; Wratten et al., 1995) and herbivorous (Annis and O’Keeffe, 1984) arthropods, it was hypothesised that the addition of pollen to the diet of Argentine stem weevil would increase the fitness of the weevil (i.e., the second trophic level), and thereby enhance the fitness of the parasitoid, *M. hyperodae* (i.e., the third trophic level). Conversely, for a deleterious food such as ryegrass with endophyte given to the weevil, the opposite effect was hypothesised.

Interactions between herbivores and their host plants and with their natural enemies can only be understood when considered within a tri-trophic context (Price et al., 1980). The addition of different diets to the second trophic level can produce a theoretical combination of no, positive or negative effects in both the second and the third trophic level, via changes in
fitness and/or survival that could affect the host’s and subsequently the parasitoid’s fitness and/or survival (Table 3).

Table 3. Combination of possible effects on the survival and/or fitness of host and parasitoid produced by changes in the host’s diet (i.e., -- = no effect, † = positive effect, ‡ = negative effect)

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<th>Host (weevil)</th>
<th>Parasitoid wasp</th>
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Chapter 2 provided evidence that Argentine stem weevil feeds deliberately on specific types of pollen in the field, and this was associated with a higher rate of gonad development (i.e., the number of individuals with sexually-mature gonads; positive effect). In the manipulative experiment described in Chapter 3, no effect of host diet on parasitoid fitness was detected. This may have been because the increase in weevil gonad development previously found to be associated with pollen was countered by a decrease in gonad development due to diapause. Argentine stem weevil diapause is associated with atrophy of the gonads, cessation of ovulation and resorption of terminal oocytes (Barker et al., 1988b). Diapause may therefore have obscured the effect of diet on weevil fitness and/or survival. Moreover, by causing female weevils to become reproductively immature during diapause (independent of their dietary treatment), possible interactions between weevil reproductive status, weevil diet and parasitoid fitness could have been suppressed.

The negative effects of the endophyte diet on the host’s gonad development were apparently compounded by diapause. A ‘false’ negative effect in the gonad development condition is discounted though, since the ryegrass-only treatment enabled the effect of endophyte on gonad development to be isolated. Also the survival of weevils fed ryegrass with endophyte was significantly decreased in this treatment, as was expected. However, no negative effects were observed on fitness of the parasitoids that emerged from weevils fed ryegrass with endophyte. This is encouraging from an integrated pest management point of view, since the potential fecundity (i.e., egg load) and body size (i.e., tibia length of hind legs) of the parasitoid are not affected by the addition of ryegrass with endophyte to the weevil’s diet.
This means that biological control and plant resistance strategies can work in a complementary way, supporting the ideas of van Emden (1990). However, it also means that, based on the results of the present manipulative experiment (Chapter 3), the addition of ‘resource subsidies’ (Gurr and Wratten, 2000) such as pollen, available to the second trophic level appears to have little scope for manipulating M. hyperodae fitness via the host diet. Although the concept of benefiting the third trophic level with resource subsidies made available to the second level remains unproven, it is potentially very worthwhile and warrants further research.

**IMPLICATIONS FOR BIOLOGICAL CONTROL**

Conservation biological control involves the manipulation of the environment (i.e., resource subsidies) to enhance the survival, fecundity, longevity, and change the behaviour of natural enemies to increase their effectiveness, and to provide shelter from adverse environmental conditions (i.e., “top – down” effects; Root, 1973; Landis et al., 2000). The mechanism studied in the present experiments, however, involves “bottom – up – top – down” effects, where ‘top – down’ are a consequence of “bottom – up” effects. An enhancement of fitness/survival of the third trophic level is theoretically made via an enhanced fitness/survival of the second trophic level. However, the benefit to the pest due to an increase in fitness of the second trophic level should be more than counteracted by the effects on the parasitoid population. In this way, the net benefit would be in favour of the third trophic level. Parasitoids with higher egg loads would be potentially good candidates to achieve higher parasitism rates because, based on mathematical models, the degree of manipulation needed to achieve a given reduction in prey populations is largely determined by the enemy’s potential reproductive rate (Kean et al., 2003). Consequently, the present mechanism has the potential to lead to the suggestion of a new way for enhancing parasitoid fitness, that is, ‘indirect’ conservation biocontrol.
Selectivity issues

The manipulation of crop and non-crop plants, without a detailed understanding of the interactions of the trophic levels involved can produce counterproductive or unexpected results (Barbosa and Wratten, 1998). Undesirable positive effects on the second trophic level may result only if a feature benefits the target pest or another herbivore (Gurr et al., 2000) and not the parasitoid. For example, flowers of coriander enhanced fecundity and longevity of C. koehleri, the parasitoid of the potato moth, *P. operculella*. However, the moths were also able to feed on these flowers, which enhanced the second trophic level to a degree where no advantage was gained by the addition of “resource subsidies” (Baggen and Gurr, 1998). Undesirable effects on the second trophic level may be avoided by the use of “selective food plants” (Baggen et al., 1999; Gurr et al., 2000b).

Buckwheat is being investigated for conservation biological control of Argentine stem weevil because it increases the fitness of *M. hyperodae* adults (Vattala et al., 2004). The low consumption of buckwheat pollen by Argentine stem weevils in the present laboratory experiment (Chapter 3), together with the lower buckwheat pollen availability in the field compared with that in the laboratory, suggests that buckwheat could be well suited for use in the Argentine stem weevil – *M. hyperodae* system. However, further laboratory and fieldwork is needed to investigate the possibility of using buckwheat as a selective plant in conservation biological control of this pest. Given that consumption of just a few pollen grains (i.e., 1 to 10 grains) can significantly increase the proportion of Argentine stem weevils with mature gonads, it is essential to be cautious in screening resource subsidies for conservation biological control in this pest – parasitoid system.

IS THERE ROOM FOR IMPROVEMENT?

Host nutrition is likely to have an effect on the reproductive success of their parasitoids (Beckage and Riddiford, 1983; Harvey et al., 1995; Jervis et al., 2001). However, based on the results obtained in the present manipulative experiment (Chapter 3), the diet of the Argentine stem weevil did not influence the body size or the egg load of *M. hyperodae*, and several possible explanations for this were discussed in Chapter 3.

If it were possible to increase the egg load of *M. hyperodae*, either in the laboratory or in the field, could potential fecundity (i.e., egg load) be translated into realised fecundity (i.e.,
parasitism rates)? Alternatively, would it be better to try to manipulate other components of *M. hyperodae* fitness?

It has been speculated that, in its native South America, *M. hyperodae* spends a considerable amount of time searching for sparsely distributed weevils (McNeill *et al.*, 1996). It may be expected that *M. hyperodae* became adapted in South America to live just long enough as an adult to lay all, or nearly all, of its eggs (i.e., so that its realised fecundity is similar to its potential fecundity). However, under New Zealand conditions, *M. hyperodae* females become egg limited relatively frequently (Phillips *et al.*, 1998; Phillips, personal communication, February 2005). This is because *M. hyperodae* is a pro-ovigenic species (Goldson *et al.*, 1995; Phillips, 1998), so its lifetime complement of eggs is set at emergence from the host. This can limit parasitoid fecundity if hosts are abundant (Heimpel and Rosenheim, 1997), because the total set of eggs can be laid before death (Phillips *et al.*, 1998). In fact, approximately 41% of the parasitoid’s lifespan occurred after cessation of oviposition under laboratory conditions (Goldson *et al.*, 1995). This (including larviposition) can also occur in herbivorous insects such as aphids, and there have been a number of ecological reasons proposed for this (Dixon and Wratten, 1971).

Several researchers have developed models that have considered the importance and risk of egg limitation in pro-ovigenic parasitoids in the field (Getz and Mills, 1996; Heimpel and Rosenheim, 1997; Ellers *et al.*, 2000; Ellers and Jervis, 2003; Kean *et al.*, 2003). A consensus seems to be emerging that parasitoid fitness can be limited by either egg supply or the time available for locating hosts (Heimpel and Rosenheim, 1997). In fact, models have predicted that increasing the longevity or fecundity of a parasitoid that becomes egg-limited in the field would have a little effect on how many hosts are attacked; however, they would have a higher effect on the parasitoid’s searching efficiency (Kean *et al.*, 2003). In addition, parasitoid searching efficiency has a higher influence on host density than does its fecundity; however, fecundity may be far more amenable to enhancement compared with searching efficiency (Kean *et al.*, 2003). In this way, influencing potential fecundity may not be the only way to enhance the fitness of a parasitoid, but certainly it is on the ‘right track’. However, as the philosopher G.S. Yule said: ‘Logic and mathematics are fine as long as they are on the right track’. So more research is certainly needed on the value of enhancing parasitoid quality in biological control.
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